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An Analysis of the Case For Producing Anthrax Vaccine in Australia

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Abstract

Anthrax represents a risk to Australian livestock industries through loss of market access. This project was undertaken to determine whether local manufacture of anthrax vaccine on land provided by the NSW Government at EMAI is an appropriate response to the proposition that a more secure supply of anthrax vaccine is required.

The risks to vaccine supply are currently manufacturing interruption, business decisions, transport disruption, regulatory intervention and insufficient supply. Local production, by itself, would not substantially reduce these risks. However, a second source of supply or establishment of a vaccine bank either alone or in conjunction with local production would be more effective at attenuating these risks to supply.

The cost of establishing local production, including a vaccine bank of a year's supply, is estimated at $2.6m. Production costs, not including cost of capital, are estimated to be $0.88 - $1.76 per cattle dose.

An alternative to local vaccine production or other options may be establishment of a vaccine bank in conjunction with preparation for local production that does not proceed unless there is a significant disruption to the current source of supply.

Executive Summary

Anthrax has been part of Australian agriculture for over 150 years. Once a cause of substantial losses, the usual outbreaks these days involve a handful of deaths in known infected regions, with control measures well developed. Vaccination is integral to controlling and preventing outbreaks although antibiotics, carcase disposal and decontamination are also important.

The main threat to Australia from the disease is the risk of loss of market access, given reactions by Asian markets to recent outbreaks. Some argue that bioterrorism against livestock also represents a threat.

In 1997 a significant outbreak of anthrax occurred in Victoria at a time when local production of anthrax vaccine had ceased and an alternative source was not yet available. Although a replacement was found, concerns about security of supply plus a substantial increase in the price of the imported vaccine led the Commonwealth Chief Veterinary Officer to establish an Anthrax Reference Group, from which a sub-group (the Industry Working Party or IWP) was formed from representatives of industry peak bodies, to explore options for achieving a more secure supply of vaccine at an “affordable” price.

Following an unsuccessful international search, in 2001 the IWP gained the agreement of the NSW Government to allow establishment of a vaccine production facility at the Elizabeth MacArthur Agricultural Institute (EMAI) on the outskirts of Sydney, on land provided rent-free. The IWP plan envisages industry funding to establish the facility, with the vaccine sold at cost of production not including the cost of capital.

Aspects of the feasibility of the proposal have twice been investigated by Dr Peter Claxton. This current project was undertaken to determine whether the proposal is an appropriate response to the issues surrounding anthrax in Australia, given other feasible options that might be available and, assuming local manufacture, to examine options, implications, and how local manufacture should be achieved.

Annual demand for anthrax vaccine has increased from about 250,000 to 400,000 cattle doses over the last 10 years, mainly attributable to use by feedlots. Demand was substantially higher in the mid 1980s due to use in live export sheep but lower in earlier decades. Few grazing livestock are vaccinated except in the face of an imminent threat. Historical reports suggest this may always have been the case.

The 1997 outbreak in Victoria involved the use of 79,000 vaccine doses and probably represents the upper end of outbreak scenarios. Terrorist scenarios might amount to similar demand in several locations simultaneously.

Australia’s only anthrax vaccine is imported from the USA and sold by Fort Dodge. The same manufacturer is also the sole supplier for the USA and Canada. Fort Dodge purchases vaccine on an arms-length basis and has no long-term supply agreement. The risks to supply associated with this include manufacturing interruption, business decisions, transport disruption, regulatory intervention and insufficient supply.

A more secure supply of anthrax vaccine would be one in which many of these risks do not apply. This could be achieved by means of a second source of supply, a vaccine bank of sufficient size, or

both. Local production would not, by itself, significantly increase security of supply. An analysis is shown in the following table.

	Vaccine Bank	Second Source	Local Production	Local Production Plus Vaccine Bank	Second Source Plus Vaccine Bank
Production risk	Largely removed*	Largely removed	No change	Largely removed	Removed
Business risk	Largely removed*	Largely removed	No change	Largely removed	Removed
International transport risk	Removed**	Largely removed	Removed	Removed	Removed
Foreign regulatory risk	Removed	Removed	Removed	Removed	Removed
Risk of inadequate supply	Largely removed*	Largely removed	No change	Largely removed	Removed

* Subject to holding sufficient stock.

** Assumes the vaccine bank is held in Australia.

If achievable on commercial terms, a second source of supply would provide the greatest risk reduction for the least cost, as shown below. Maintenance of two sources may not be feasible unless they are at a similar price.

	Option	Cost To Establish	Risk Reduction	Details
1	Second Source	Low	High	Costs involved in locating a second source probably borne by industry. A second source is assumed to be on commercial terms.
2	Vaccine Bank	Moderate	High	Inventory and storage costs would probably be paid by industry.
3	Local Production	High	Low	Establishment of the facility would be at industry expense.
4	Local Production Plus Vaccine Bank	High	High	Equivalent to vaccine bank. Assumes local production is the only source.
5	Second Source Plus Vaccine Bank	Moderate	Very high	This is the combined effect of 1 and 2.

The market within Australia is too small to sustain commercial production and there are no domestic vaccine manufacturers willing to produce anthrax vaccine in existing facilities, although Fort Dodge is prepared to help establish a vaccine bank. However, internationally the market for anthrax vaccine functions reasonably well.

The search by the IWP for a second source of vaccine was not successful largely due to an inability to meet Australian regulatory and quarantine requirements. However, its inquiries were not exhaustive and there is a possibility that other suitable manufacturers could still be found among the 15-20 possibilities.

The IWP also sought to secure anthrax vaccine at a lower price. Although the price (currently about 92 cents per cattle dose ex Fort Dodge) is several times higher than immediately prior to cessation of local production, in real terms it is about double what it was in 1925. There is no evidence of monopoly pricing by Fort Dodge.

Based on price and demand history, the claim that a lower price for anthrax vaccine would lead to a major increase in demand for grazing livestock cannot be sustained. Nonetheless, a promotional campaign focusing on the threat to market access may have that effect.

The proposal to establish local production at EMAI has been examined on the basis that Sterne strain vaccine would be produced containing at least $10x10^6$ spores per ml, registered with the APVMA, in a facility that complies with the latest code of Good Manufacturing Practice (GMP). A multi-purpose facility is an option. A vaccine bank of one year's supply is included.

As a location, EMAI appears suitable with the possible exception of the animal house, although with batch potency testing in guinea pigs apparently unnecessary this is probably not relevant. While there are considerable challenges, no insurmountable barriers to the production and registration of vaccine have been identified.

Nonetheless, while the technology of production of the Sterne strain anthrax vaccine has barely changed since 1937, the environment in which the vaccine is manufactured bears little resemblance. Moreover, further changes to the GMP code are imminent that will significantly increase the costs of obtaining and maintaining APVMA certification.

The establishment of a multi-purpose facility to produce anthrax vaccine at EMAI is estimated to cost about $2.6 million, comprising plant costs of $1.6 million and commissioning costs of $0.9 million.

Up to six personnel are required. In increasing degree of feasibility, options include the use of EMAI staff, experienced external staff or involvement of a third party vaccine manufacturer. In each case, experienced manufacturing and quality assurance managers would be essential.

The cost of producing 450,000 doses of anthrax vaccine per year in the plant is estimated to be $0.88 - $1.76 per cattle dose, including all costs other than cost of capital but with no batch failures. There is potential for this to be significantly higher, particularly initially, unless production efficiency and batch success are optimised. Using a lower spore count, similar to the Fort Dodge product, would reduce the cost by about 15 cents a dose.

Major recurring costs include product liability insurance and depreciation, which in total add over 40 cents per dose. More than two years would be required from commencement to availability of legally saleable vaccine.

If the vaccine were to be sold at cost as calculated, Fort Dodge may not withdraw from the market. This would contribute to increased security of supply but may further increase the cost due to lower sales.

The organisation would require a system for receiving and despatching orders, managing funds and responding to complaints. A means of ordering via a consortium member such as ALFA or NSW

Farmers is an option, with distribution via rural merchandise retailers. Beyond advising how to order vaccine, there is no necessity for marketing.

Capital funding options include livestock industry levies, stakeholder cost sharing and government. Unless funded by current levy recipients (eg MLA or AWI), levy funding would require legislation. A commercial partner is a possibility if the vaccine was sold on commercial terms and the facility could be used for other purposes.

A proprietary company limited by shares would meet the entity requirements of anticipated participants while also offering significant flexibility for the future. A board of three to six directors would be sufficient, representing shareholders and other interests.

There are several options apart from commencing local vaccine production.

One is to resume the international search for a second source of vaccine. Although the prospects may not be great, success would provide an effective and economical solution.

A variation on that is to wait until human anthrax vaccine development delivers a recombinant product that can be imported into Australia. Given recent developments and the level of resources now committed to preventing anthrax in humans, particularly in the USA, this is considered likely to emerge in the foreseeable future.

A vaccine bank would increase security of supply and may be the most feasible option in terms of risk reduction relative to cost, although it would always be ultimately dependent on a reliable and acceptable source of supply.

There may also be options for reducing market risk. If cattle were vaccinated prior to entering a feedlot, for example, any incubating cases would occur on the farm rather than the feedlot. This could be achieved by the feedlot industry introducing into its code of conduct an obligatory requirement for quarantine with or without anthrax vaccination two weeks prior to feedlot entry.

Other markets considered particularly vulnerable to closure due to anthrax could be similarly identified and a targeted regime of vaccination introduced.

Finally, a variation on the proposal to establish a plant at EMAI immediately would be to make detailed preparations for construction but delay commencement until triggered by certain specific events. Broadly, these could include anything that disrupted the current source of supply with little prospect of short-term restoration or identifying an alternative.

Whether local production of anthrax vaccine is an appropriate response to the issues surrounding the disease involves not only cost and feasibility, but also consideration of whether the threat to trade can be reduced in other ways, whether anthrax is sufficiently significant relative to other diseases for which there may be no vaccine locally produced, and whether the bioterrorism scenarios are valid. These are matters of judgement with limited objective information to provide assistance.

If it is considered that Australia requires greater security of supply of anthrax vaccine, our investigations suggest this would not be achieved by local production alone. Local production combined with a vaccine bank would be effective but no more so than a vaccine bank based on an overseas source.

A vaccine bank combined with a contingency plan to build a vaccine plant locally if there is a serious disruption to supply would achieve virtually the same result provided the bank held enough stock to cover the period before production commenced.

If it is considered that Australia requires a more economical anthrax vaccine, our investigations indicate this would not be achieved by local production, at least within the assumptions that we have applied. A greater level of subsidy than envisaged would be necessary for this to occur. A second international source of supply, if obtainable, is probably more likely to contribute to a lower price.

If local manufacture were to proceed we are confident that, although the challenges should not be underestimated, there are no insurmountable barriers to establishing a plant at EMAI to produce anthrax vaccine.

However, given the rapid developments in human anthrax vaccine development now occurring, it may be that the plant would be redundant within ten years.

Contents

1 Background To This Project

Anthrax vaccines have been available in Australia since the late 19th century. The first effective single dose anthrax vaccine (the McGarvie-Smith Gunn vaccine) is claimed as an Australian invention.

For much of the 20th century the Australian anthrax vaccine was produced by the McGarvie-Smith Institute, a corporation established by the NSW Government to implement the wishes of the late John McGarvie Smith who in 1918 donated to the Government (along with a substantial amount of money) his discovery of a stable anthrax vaccine that gave protection with a single inoculation.

Around 1960 the Institute transferred its know-how and equipment to Commonwealth Serum Laboratories and Arthur Webster Pty Ltd, both of which made the vaccine until the late 1980s when Arthur Webster began producing the vaccine for CSL.

Arthur Webster was acquired by American Cyanamid in 1993 and then taken over by Fort Dodge (parent company Wyeth, formerly American Home Products) in 1994.

In 1996 CSL, which was still selling vaccine produced by Fort Dodge, was approached by Fort Dodge to resume vaccine manufacturing. Fort Dodge wished to cease anthrax vaccine production to use its facilities for other purposes.

CSL agreed to recommence production and worked out a timetable. Fort Dodge built up almost a year's supply of the vaccine to cover the period until CSL expected to have stocks available and then shut down production and disposed of equipment.

Commencing in early January 1997 there was a significant anthrax outbreak centred on Tatura in Victoria[1], the control of which consumed all the locally produced vaccine. Faced with the prospect of no available supplies of vaccine the Commonwealth Government conducted an international search for an alternative source. Colorado Serum Company in the United States was identified as acceptable, based largely on quarantine grounds. Fort Dodge then began importing vaccine from the company under permit.

After the outbreak Fort Dodge continued to supply the US-sourced product, applying for and receiving full registration from APVMA in 2003. It remains the only livestock anthrax vaccine available in Australia.

In early 1998, concerns about the vulnerability of vaccine supply, motivated by the shortage during the Tatura outbreak and reliance on an imported source, together with complaints about price rises of the imported vaccine, prompted the Commonwealth Chief Veterinary Officer to establish an Anthrax Reference Group.

Members of the group initially included representatives of the Chief Veterinary Officers of the Commonwealth, Victoria and NSW, representatives from AFFA (now DAFF), the NSW Farmers Association, Victorian Farmers Federation, Australian Lot Feeders Association, and State Council of Rural Lands Protection Boards.

The Group focused on two key objectives:

1. To establish a more secure source of supply of anthrax vaccine, based on the proposition that the current source was not secure.
2. To make anthrax vaccine available at a "more affordable" price, which essentially meant a price closer to that applying prior to the introduction of the imported product.

[1] Galvin 1997; White 1998; Turner et al 1999a,b; White 1998

In July 1998 CSL confirmed to the Anthrax Reference Group that it intended to resume vaccine production by mid 1999 and was expecting to sell the vaccine at no profit for around 30-50 cents per dose[2].

However, in September 1998 the company advised the Group[3] that it no longer had a facility available for anthrax production. The company's only option, it said, would be to establish a mobile plant. Unless the Government underwrote the cost of the plant, the company anticipated the cost of the vaccine would be of the order of the imported vaccine.

That resulted in an Industry Working Party (IWP) of the Reference Group led by Peter Carter (representing NSW Farmers), exploring options for obtaining an alternative vaccine source.

Efforts were directed both at identifying a source outside the country and recommencing local production.

Local manufacturers were approached to determine if they were able or willing to manufacture an anthrax vaccine for the Australian market. None was able to do so, or prepared to do so, unless the capital cost of establishing or upgrading plant and equipment was covered.

In 1999 Fort Dodge apparently offered to import and distribute vaccine on a fee for service basis. However, this was not accepted by the IWP.

Several overseas manufacturers were investigated, with suppliers in Spain and the Czech Republic looking hopeful for a time. However, APVMA and AQIS requirements meant that each of these manufacturing facilities would have required significant upgrading of plant and equipment. The cost of this would either have to be met by Australia or incorporated into the product price.

The IWP deemed the payment of significant industry funds to corporations, particularly overseas ones, to be unacceptable.

In 2001 the IWP gained the agreement of NSW Agriculture to manufacture vaccine within the grounds of the Elizabeth McArthur Agricultural Institute (EMAI). The EMAI is Australia's national reference laboratory for anthrax.

The terms of that agreement, as outlined in letters from the NSW Minister for Primary Industries to Peter Carter, indicate that land could be provided for such a purpose while staff may be utilised on a cost-recovery basis.

NSW Agriculture has twice commissioned studies by Dr Peter Claxton into the EMAI proposal. In June 2002 Dr Claxton concluded that a production cost ranging from 13 to 87 cents per cattle dose was possible in a facility estimated to cost $1.5 million. In calculating this range of production costs it was assumed that a cattle dose contained 10 million spores and that every batch of bulk antigen met specifications (ie there was no loss of batches).

The main cost exclusions, which need to be added to the cost of goods, were identified at the time as indemnity insurance, regulatory fees and security. Other significant components of cost to be added include production wastage (failed batches), quality control (especially the cost of potency testing and safety testing in animals), maintenance costs and other annually recurring costs.

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[2] Meeting Report, Anthrax Vaccination Meeting. 2 July 1998

[3] Meeting Report, Anthrax Reference Group. 25 September 1998.

In January 2004 Dr Claxton assessed the risks involved in the EMAI proposal, primarily in respect of issues relating to product indemnity insurance. He estimated cover of $20 million would attract an initial premium of $100-120,000, although this should fall in subsequent years.

The EMAI proposal remains the IWP's preferred option to provide a secure supply of anthrax vaccine. The expectation is that the capital cost would be shared among industry bodies in a similar manner to the Emergency Animal Disease Preparedness cost sharing agreement, while the vaccine would be sold "at cost" (i.e. cost of production) to livestock producers. It also would prefer the vaccine to be distributed via non-profit channels.

Unresolved issues remain with respect to the proposal and there are continuing reservations within State and Commonwealth governments.

In 2003 the Primary Industries Ministerial Council noted that all options for ensuring a reliable supply of anthrax vaccine should be investigated[4]. In 2004 the PIMC concluded that greater reliability of supply of anthrax vaccine could be assured by either a local manufacturing facility or a vaccine stockpile held in Australia[5], if it was deemed necessary.

It requested Animal Health Australia to investigate funding options for establishing an interim vaccine reserve to secure supply, pending final determination on local manufacture.

It is generally understood that the establishment of a vaccine facility will not proceed unless there is substantial support from livestock producers through Peak Councils, State government and possibly the Commonwealth government. To this end the IWP requested Meat and Livestock Australia to commission this study to review and analyse the case for producing anthrax vaccine in Australia so that a final determination can be made.

The study was undertaken by Baron Strategic Services, supported by Advanced Veterinary Therapeutics.

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[4] PIMC Resolution 4.8, 2 October 2003

[5] PIMC Resolution 5.16, 19 May 2004

2 Objectives

The objective of this project was to provide a thorough and critical analysis from which industry and government could resolve:

- Whether a plant for manufacturing vaccine in Australia is an appropriate response to the issues surrounding anthrax in Australia, given other feasible options that might be available.

- Assuming local manufacture, what are the options and implications for pursuing this option and how should local manufacture be achieved.

3 Anthrax In Context

Summary

Anthrax has caused livestock deaths in Australia for over 150 years. The usual outbreaks these days involve a handful of deaths in known infected regions. Larger outbreaks are uncommon and control measures well developed.

The risk to export market access from outbreaks is the main threat presented to Australia by the disease.

The potential use of anthrax against livestock has also been raised as a terrorist threat following its use against humans in the USA.

3.1 The Disease in Australia

Anthrax is a peracute to subacute infectious bacterial disease that can affect both animals and humans. Cattle are particularly susceptible to anthrax and the course of disease from first clinical signs to death can be as little as 2 hours.

Caused by the organism *Bacillus anthracis*, anthrax occurs almost worldwide in generally well-known infected areas[6].

The disease has been recognised in Australia for over 150 years as a cause of sudden death in farm animals, particularly sheep and cattle but occasionally horses, goats and pigs. Animal Health Australia records its history as follows[7]:

> *Anthrax is believed to have been introduced into Australia around the middle of the 19th century. It probably first occurred in 1847, at Leppington, approximately 40 km south-west of Sydney where it was known as Cumberland disease. It is believed that infection was probably introduced in imported contaminated bonemeal, manuring with powered bone being a recommended procedure for pastures and crops at the time.*
>
> *From the initial focus at Leppington, anthrax was spread by travelling stock. Movement of large numbers of livestock resulted in spread throughout practically all the sheep-raising areas of New South Wales and into southern Queensland with considerable losses experienced.*
>
> *Anthrax was first recognised in Victoria in 1886, at Warrnambool, from where it spread over most of the western district and later to southern and central Victoria. The initial Victorian outbreak is believed to have occurred independently of spread in New South Wales, although later distribution in northern Victoria was probably associated with movement of infected stock over the border.*
>
> *Occasional outbreaks were seen in the States of South Australia and Tasmania. However, there has been no evidence of the disease since the last reported outbreaks in South Australia (1914) and Tasmania (1933), and these States are now considered free. Queensland most recently had cases of anthrax in 2002. Anthrax was diagnosed in Western*

[6] Hugh-Jones 1998; WHOCC 2005 (http://www.vetmed.lsu.edu/whocc/mp_world.htm)

[7] Animal Health Australia 2005

> *Australia in 1994 but is now considered free. The disease has never been reported in the Northern Territory.*
>
> *Vaccination was adopted in 1890 and this was highly successful in controlling the disease. Anthrax is now quite uncommon in Australia and clinical cases of the disease are seen only sporadically. The majority of outbreaks occur in sheep, with some in cattle and occasionally pigs. Rarely, cases occur in goats and horses.*

Australia has known infected areas in New South Wales and Victoria where the organism persists as spores in the soil. These areas tend to have a neutral to alkaline soil (pH 7 to 8) and to be on flood plains along waterways. Infective spores can remain dormant for decades although the factors governing their re-emergence to cause disease are far from fully understood[8]. According to the Australian National Animal Health Information System, there were a total of 148 incidents of anthrax reported in Australia in the 13 years from 1992 to 2004, an average of 11 incidents each year but varying from 2 reports (1999 and 2000) to a peak of 21 (1995 and 1997)[9].

In Victoria the disease occurs sporadically with most outbreaks located in the northern and north-eastern parts of the State. Small foci have also been reported at Werribee and west Gippsland. Dairy cattle are the most commonly affected animals, with sheep and beef cattle rarely affected.

In New South Wales, anthrax occurs sporadically and affects both sheep and cattle, though generally in separate incidents. It tends to occur in a well defined region in the centre of the State on the plains in the south and on the plains, low tablelands and adjacent slopes in the north, in areas where annual rainfall is 250–500 mm. The disease usually has a distinctly seasonal distribution, with most cases occurring during summer. Incidents are more common in seasons that are drier than average, often following a period of wet or humid weather. A comprehensive study of anthrax in livestock in NSW found that over the period 1960 to 1979, anthrax preferentially affected cattle in the southern reaches of the anthrax belt while sheep and cattle were similarly affected in the north[10]. Over the last 80 years the annual average number of anthrax incidents in livestock reported in NSW approximates 20 with no significant trends over time[11].

Anthrax occurs only rarely in other parts of Australia. In Queensland, it was confirmed in one cow on a large extensive grazing property near Rockhampton in September 1993, the first reported case in that State for more than 50 years[12]. In 2002, cases occurred on two farms in southern Queensland[13].

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[8] Whitford 1984; de Vos 1994; Turnbull 1998; Turner 1999b

[9] http://www.animalhealthaustralia.com.au/programs/adsp/nahis/nahis_home.cfm

[10] Kennedy 1979

[11] Wise 1993

[12] Animal Health Australia 2005.

[13] Berry 2001

In Western Australia, anthrax was confirmed in cattle on two farms in the Walpole district in the far southwest of the State in March 1994, the first ever reported incident in the State[14]. Since then cases were diagnosed in cattle on a third farm in the same district. Quarantine and other control measures were immediately applied once the diagnosis was established and no further cases have occurred. It is pertinent to note that it took several months to confirm the diagnosis of anthrax in the WA incident, with samples sent to NSW for assistance. This highlights the value of readily available reliable, specific and sensitive diagnostic tests.

Losses from anthrax incidents are generally low and can be controlled with appropriate carcase disposal (incineration recommended), quarantine and vaccination. Rarely, as occurred in the Tatura district of Victoria in 1997, outbreaks can be more significant and the cost to industry greater, though outbreaks can generally be quickly and effectively controlled.

The epidemiology of anthrax today is significantly different from that experienced in the late 19th century. While numerous outbreaks with substantial animal mortality were common 120 years ago, the usual anthrax incident in recent decades has involved one or two deaths at affected premises. These appear to reflect a point source with low potential for spread. If the primary or index case is identified, the carcase incinerated and the environment disinfected, then no further cases are likely. It is also unusual to experience repeated incidents on the same premise. With or without vaccination, sporadic point source incidents are likely to be readily and effectively controlled.

It is important to recognise that anthrax is not contagious between affected livestock. In contemporary Australia, infection is nearly always a consequence of ingestion of spores released from the carcase (recent or from a former burial site) of an affected animal.

3.2 Dealing With Anthrax Incidents.

Measures taken to deal with occurrences of anthrax in farm animals are well developed and designed to limit and control disease incidents, protect domestic and export markets for livestock and their products, and safeguard public health.

Australian veterinary authorities maintain emergency plans, based on AUSVETPLAN[15], for the control of a large-scale anthrax outbreak.

When anthrax is suspected or diagnosed on a property, the following control measures are put in place:

- The property is quarantined, preventing all movement of stock to prevent spread.
- Prompt vaccination and/or treatment of exposed / at risk livestock. Vaccination generally includes surrounding properties.
- Safe disposal of carcases (preferably by burning) and decontamination of the environment at the site(s) of deaths to contain spores and prevent spread of infection.
- An epidemiological investigation is conducted to identify the index case, establish the source, and trace livestock movement on and off the farms.

[14] Forshaw et al 1996

[15] Animal Health Australia 2005

- All movements of stock off the property for the previous 20 days are traced and quarantined and/or vaccinated according to risk assessment.

- Quarantine remains in force for 20 days after the last death or 42 days after vaccination, which ever is later, to comply with international trade regulations[16].

- To ensure the safety of livestock products, potentially infected livestock and livestock products are prevented from being processed for human or animal consumption or industrial use.

- Advice is provided to people handling livestock and liaison initiated with public health authorities in the event of anthrax exposure to reduce the risk of human infection and to ensure appropriate treatment is provided if necessary.

- Industry awareness of anthrax is increased by providing advice to appropriate livestock production, transport, selling and processing sectors.

Livestock owners and veterinarians in areas of Australia where anthrax occurs are reminded seasonally to be alert to signs of the disease and to report, without delay, suspicious cases to veterinary authorities. The Victorian government holds stocks of anthrax vaccine for rapid use in an incident.

3.3 Anthrax In Humans

There are three different forms of anthrax recognised in humans, according to the route of exposure[17].

The skin form of the disease (cutaneous anthrax) is most common worldwide. It is usually contracted after the entry of spores to the body through cuts and abrasions via contaminated soil, infected live animals, contaminated animal carcasses or animal products such as wool. Veterinarians undertaking autopsies of stock are particularly at risk with six cases of cutaneous anthrax reported in the US between 1950 and 2001[18].

In Australia, only the skin form of anthrax in people has ever been recorded. This responds well to treatment and is the mildest form of the disease. The mortality rate in untreated cases is between 5 and 20 percent.

The inhalation form of anthrax is acquired through inhaling a significant number of aerosolised anthrax spores resulting in pulmonary anthrax which is extremely difficult to treat and usually fatal. Until recent times and the development and use of weaponised spores of *Bacillus anthracis*, pulmonary anthrax was primarily an occupational hazard particularly amongst wool handlers and tanners handling infected skins. Woolsorters' disease was first shown to be caused by the agent of anthrax in 1879 by John Henry Bell[19].

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[16] OIE 2005

[17] Dixon et al 1999

[18] Bales et al 2002

[19] Cunningham 1976

The gastrointestinal form is acquired through the ingestion of insufficiently cooked meat that has been contaminated with anthrax spores. If the infection becomes generalised, the death rate is 25 to 60 percent. The first significant report of intestinal anthrax was that of an epidemic in Saint-Domingue (now Haiti) in 1770 that claimed the lives of 15,000 people[20]. Sporadic cases of intestinal anthrax continue to be reported from a number of regions of the world.

In earlier times the disease was an occupational hazard of working with livestock, as spores would get stuck in the wool/skin of animals. The workers often developed skin infections and sometimes inhaled spores if they were in a closed environment.

Anthrax continues to cause thousands of deaths in people in developing countries around the world, largely as a result of the consumption of insufficiently cooked carcasses of animals that have died from anthrax.

3.4 Anthrax As A Terrorist Threat

Anthrax has been regarded as a potential biological weapon for decades[21].

A number of suspected uses of anthrax against transportation animals by the German government during World War I were alleged, but not well documented.

Japan, Great Britain, and the United States all conducted research into the use of anthrax as a weapon in World War II. While no combatants used anthrax, it was tested on animals and the Japanese used it on allied and Chinese prisoners. Weapon delivery systems were also developed.

In response to the fear that German rockets would be loaded with biological agents, Winston Churchill ordered preparations to retaliate by dropping anthrax-contaminated cattle cakes in the German countryside to affect beef supply.

During the Cold War the former Soviet Union developed a biological research programme. The accidental release of anthrax from a secret bio-weapons research facility in Sverdlovsk, USSR resulted in the death of 66 of 77 diagnosed cases[22].

With the establishment of a biological weapons research facility at al-Hakim, Iraq, in 1988, concerns over the use of anthrax during the Gulf War resulted in US troops being vaccinated. It is not believed that anthrax was used by the Iraqi armed forces.

In 1993, the Aum Shrinkyo cult attempted to release anthrax spores in downtown Tokyo one month prior to the infamous and widely reported sarin nerve gas attack. This incident marked the first instance of anthrax being used as a weapon against a civilian population, although the strain of anthrax used in this case was not pathogenic (being the avirulent nonencapsulated animal vaccine Sterne strain)[23].

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[20] Morens 2002

[21] Bhalla & Warheit 2004

[22] Meselson et al 1994

[23] Keim et al 2001

Within the United States the potential use of anthrax by terrorists became a major concern in the late 1990s. In 1998, 60 Minutes broadcast a story concerning the potential for the use of anthrax as a weapon in major cities such as New York and Boston, based on a hypothetical case study published by the Centers for Disease Control[24].

In October 2001 the concern became reality when a number of anthrax-contaminated letters were sent through the post to media personalities and politicians[25]. This resulted in 23 people being infected, the majority of them postal workers, with five deaths from the inhalation form of the disease. Of the others, six inhalation and 12 cutaneous cases were successfully treated while thousands of others were treated on the basis of known or suspected exposure.

Aside from the extensive security measures that followed, this also prompted a massive research effort to develop a more effective human vaccine, effective treatments, improved decontamination procedures and rapid diagnostic methods – all technologies and methodologies with potential application to livestock disease management.

3.5 The Risk To Market Access

Anthrax represents a risk to the livestock industries because of its potential effect on market access[26].

This is not necessarily new. In a paper read before the Royal Society of NSW on June 3, 1891, Adrien Loir, an associate of Louis Pasteur in Australia to support the introduction of anthrax vaccination, noted the growing meat export trade and concluded that, “Is it not in the best interests of the Colonies to adopt every possible precaution to prevent European Bacteriologists from finding in Australian meat, microbes or remains of microbes in large quantities, a discovery to which the utmost publicity would be given by those interested in stopping the importation of foreign meat?”[27]

Anthrax is on both the National List of Notifiable Diseases[28] and the list of diseases notifiable to the OIE (World Organisation for Animal Health)[29]. Outbreaks must be notified to state and commonwealth animal health authorities and Australia must advise the OIE. Thus the rest of the world quickly learns of all anthrax incidents.

Loss of market access in response to an anthrax incident is not justified on scientific grounds and is contrary to OIE policy[30]. Nonetheless, as a result of the Tatura outbreak in 1997, a number of

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[24] Inglesby 1999

[25] Traeger et al 2002

[26] Ban 2000

[27] Loir 1891

[28] Agreed by Animal Health Committee, a sub-committee of the Primary Industries Standing Committee. The Chief Veterinary Officers of the Commonwealth, States, Territories and New Zealand, along with a representative from CSIRO, Biosecurity Australia and Animal Health Australia form the Committee.

[29] http://www.oie.int/eng/maladies/en_classification.htm

[30] Terrestrial Animal Health Code 2005 Chapter 2.2.1

countries imposed temporary restrictions on the import of Australian meat and dairy products. These included Indonesia, Thailand and the Philippines. Trade with Japan, Republic of Korea, Thailand, Taiwan and China (including wool) was sufficiently threatened to warrant specific reassurances.

Following an isolated case in Victoria in 2002 the Philippines Government again effectively banned the import of Australian beef. The Philippines Agricultural Secretary, Leonardo Montemayor, said: "We cannot compromise the health and safety of the Filipino people."[31]

The two countries have experienced trade disputes in the past with the Philippines angry that Australia imposed bans on the importation of Filipino pineapples and bananas on the grounds of disease. Meat is Australia's largest export earner to the Philippines.

Following the anthrax incident in Queensland in 2002 there were reports of nervousness from Japan that prompted Safe Meat[32] to provide specific reassurance.

The link between anthrax and market access is recognised by government. The communiqué from the Primary Industries Ministerial Council Meeting on 19 May 2005 included the following comment:

> Ministers noted advice that market access for meat, dairy products and other animal products can be disrupted in major outbreaks of anthrax and in sporadic cases that are not well managed.

Australia is not alone in this situation. In 2002 the import of poultry from the US was banned by Russia over concerns about anthrax. (Prior to its accession to the WTO, Russia also raised the risk of anthrax in relation to Australian wool imports.)

The value in 2003 of livestock product exports to Asian markets were as follows[33]:

Livestock Product Exports To Asia 2003 (A$m)				
	Beef	**Live cattle**	**Sheep meat**	**Wool**
Philippines	23	39		
Malaysia and Singapore	87	39	36	
Indonesia	38	203		
Japan	1,384	17	72	58
South Korea	251		2	60
Taiwan	127		32	108

[31] http://www.infarmation.com.au/common/commodities/ernstandyoung/Agri_News_385.pdf

[32] Safe Meat (http://www.safemeat.com.au) is a red meat industry partnership body comprising representatives from producers, processors, exporters and government. It was established to ensure that red meat products achieve high standards of safety and hygiene and to provide strategic direction and policy advice to the industry.

[33] Australian Commodity Statistics 2004. Australian Bureau of Agricultural and Resource Economics, Canberra.

China inc Hong Kong	15			1,175

In addition to the national situation, anthrax also represents a market access risk for individual livestock producers as a result of actions by individual abattoirs, dairy processors or supermarkets.

An abattoir that discovered a case of anthrax in its premises would suffer significant disruption. The AQIS Export Meat Manual stipulates that all slaughtering operations must cease while the case is dealt with, the carcase disposed of and decontamination procedures undertaken in accordance with prescribed requirements.

Some abattoirs are apparently quite sensitive to the risk. We were told of one establishment that, during the Tatura outbreak, had sought to identify all the farms in an area known to have ever suffered an anthrax outbreak. Although the intention was not stated, it was presumed that the abattoir anticipated either refusing to buy stock from those farms or at least imposing limitations.

The major supermarkets, Coles and Woolworths, impose quality criteria on meat suppliers that go well beyond scientific issues. It would not be unexpected for an individual producer, or a group of producers in an area, to lose access to these businesses as a result of an anthrax incident.

AUSVETPLAN[34] (Version 3.2) describes anthrax in the following terms:

> *In Australia, a major outbreak of anthrax is included as a Category 3 emergency animal disease in the Government and Livestock Industry Cost Sharing Deed In Respect of Emergency Animal Disease Responses (EAD Response Agreement).*
>
> *Category 3 diseases are emergency animal diseases that have the potential to cause significant (but generally moderate) national socioeconomic consequences through international trade losses, market disruptions involving two or more states and severe production losses to affected industries, but have minimal or no effect on human health or the environment.*
>
> *For this category, the costs will be shared 50% by governments and 50% by the relevant industries (refer to the EAD Response Agreement for details).*

[34] http://www.aahc.com.au/ausvetplan/index.htm

4 The Significance Of Vaccines

Summary

Apart from early administration of antibiotics, vaccination is the only reliable means of preventing deaths from anthrax.

A vaccine based on a modified live strain of *Bacillus anthracis*, usually the Sterne strain, has been used to vaccinate livestock since the 1930s and requires a single dose for primary vaccination.

A multi-dose non-living vaccine using a culture filtrate from the organism is used in humans, although vaccination was not widespread until military use during and following the Gulf War.

Concerns about side effects and the dose regime led to concerted research efforts that will result in a new recombinant vaccine becoming available in 2006. This has implications for future livestock vaccines.

New diagnostic technology allows the identification of contaminated environments and infected livestock that are incubating the disease. This is expected to improve control of outbreaks.

4.1 General

If infected cattle, sheep or goats are identified very early in the clinical course of anthrax they can show a dramatic response to appropriate doses of antibiotics. Asymptomatic at-risk animals that are incubating anthrax may similarly benefit greatly from antibiotic treatment. However, the sudden nature of the disease means treatment is rarely possible in animals displaying signs of disease.

Cattle veterinarians in North America routinely manage new anthrax incidents by treating in-contact and other at-risk animals with antibiotics (especially a long acting oxytetracycline injection) followed by vaccination about 10 days later, allowing systemic antibiotic concentrations to fall below those that would interfere with successful vaccination with a live spore vaccine.

The situation with humans is generally the same. The US Department of Defence advice is that antibiotics are unlikely to be effective against pulmonary infections unless commenced prior to the onset of symptoms.

By contrast, vaccination is effective in preventing anthrax. As noted by the Primary Industries Ministerial Council[35], anthrax vaccination of livestock may be undertaken in four potential situations:

1. Emergency vaccination to prevent further cases when anthrax occurs
2. Preventive vaccination to protect livestock on premises where anthrax has occurred in previous years
3. For commercial purposes – some feedlots have policies to vaccinate all cattle against anthrax, and

[35] PIMC, Resolution 4.8, 2 October 2003

4. To respond to bioterrorism.

Vaccination will prevent disease in animals on properties in which the organism is present in the soil including in uninfected animals on a property experiencing an outbreak. New cases are rarely if ever seen more than eight days after vaccination although full immunity takes 10 to 14 days to develop.

It can be difficult to distinguish between the results of vaccination campaigns which overlap with the natural termination of an outbreak. Hugh-Jones and de Vos described the natural biology of an anthrax outbreak by noting that, "as the disease passes through the population at risk, most mortalities occur on a wave-like front, with a high incidence of new cases occurring on the leading edge of the front. Behind the front, the number of cases progressively decreases, and after a few weeks only sporadic isolated mortalities are seen in the area through which the front has moved."[36].

The published evidence supporting the efficacy and safety of the Sterne vaccine has had few contributions since the original descriptions by Max Sterne himself in 1937. The efficacy and safety of the Sterne vaccine today is generally accepted on the basis of nearly 70 years of worldwide use in livestock.

While there can be no doubt that the vaccine induces a protective immune response in cattle and sheep, there is only limited information on the duration of immunity, the number of animals that do not respond to vaccination and the relationship between spore count and efficacy[37]. This paucity of data also applies to other anthrax vaccines, with few publications of duration of immunity with strain STI[38], C5[39], strain 55[40] or the Sterne strain utilising an alternative adjuvant of lanolin and liquid paraffin[41].

The vaccine originally developed and described by Sterne in 1937 had a viable spore content of 300,000 spores per dose[42] which was later increased to fall in the range 600,000 to 1.2 million spores per cattle dose[43]. It was then further increased to 10 million spores[44] and finally, 10 years later, the vaccine contained 20 million spores per ml[45]. The reasons for the progressive increase in spore count have never been reported but presumably reflected a perception of a need to

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[36] Hugh-Jones & de Vos 2002

[37] Published literature describing the Sterne vaccine is identified in Appendix 4.

[38] Bakulov et al 1987; Tsydypov et al 1989

[39] Aarabi and Sotoodehnia 1984

[40] Kolesov et al 1976; Ipatenko et al 1989, 1993

[41] Al-Dabbass et al 1986

[42] Sterne 1937b

[43] Sterne 1939a

[44] Sterne 1946

[45] Personeus et al 1956

improve vaccine efficacy. Despite the high spore counts, there are a large number of published reports describing anthrax in cattle that had been vaccinated at least two weeks previously[46], emphasising that vaccination does not provide infallible protection. Other studies of serum titres in vaccinated cattle have shown considerable variation between animals and in many cases have been unable to demonstrate antibodies to anthrax protective antigen[47].

Vaccines are also quite effective (more than 95%) in humans. However, vaccination is not routinely recommended. In Australia, vaccination is only advised for people who may be at risk of repeated exposure to anthrax spores. This includes workers in special laboratories or those who would be involved in the clean up of any areas known to be contaminated with anthrax.

The only significant exception to this is the military.

During the 1991 Gulf War, concerns that Iraq had prepared anthrax spores for use as a biological weapon motivated the US military to administer anthrax vaccine to an estimated 150,000 service members. After the war, admission by Iraq that it had indeed produced weapons containing anthrax spores confirmed fears of the potential use of anthrax as a biological weapon. In 1997 the Secretary of Defence announced plans to vaccinate all US military personnel for anthrax. Vaccinations were phased in gradually, with those in more high-risk regions given priority.

However, unsubstantiated but nonetheless widespread concerns were raised about the safety of the vaccine with claims of adverse side effects including a link to Gulf War syndrome. In 2004 the Department of Defence was prevented by injunction from continuing the vaccination program on a compulsory basis. Currently, vaccination of service personnel and their families serving overseas in designated areas (essentially Asia) is strongly encouraged but may be refused.

Australian service personnel deploying to the Middle East are also being vaccinated although those who do not accept vaccination will not be sent.

4.2 Vaccine Technology

The vaccine used in livestock is significantly different from the vaccine used in most parts of the world to vaccinate humans.

The most widely used livestock anthrax vaccine comprises viable spores of an avirulent, non-encapsulated strain of *B anthracis* suspended in glycerol saline with saponin as adjuvant. Because it is a living vaccine and successful immunisation is dependent on spore germination, concurrent administration of antibiotics is likely to inhibit the development of immunity[48].

This vaccine was developed by Sterne in 1937[49]. He derived a rough variant of virulent *B anthracis* from culture on serum agar in an elevated CO_2 atmosphere. This variant, named $34F_2$, is incapable of forming a capsule as it has lost the pX02 plasmid, which codes for capsule

[46] For example Steele & Helvig 1953; Van Ness et al 1959; Lindley 1963; Brunsdon 1971; Kaufmann et al 1973; Fox et al 1977; Kennedy 1979; Salmon & Ferrier 1991; Wise 1993; Forshaw et al 1996; Patra et al 1998; Turner et al 1999b; Noseda 2002

[47] WHO 1996, 2006; Spencer et al 1994

[48] Lee et al 1961; Webster 1973; Malovastyi 1985

[49] Sterne 1937b

formation. In Central and Eastern Europe an equivalent pX02 derivative, Strain 55, is the active ingredient of the current livestock vaccine.

Both vaccines based on unencapsulated strains are generally safe, non-pathogenic and provoke protective immunity in vaccinated animals. However, it should be noted that some animal species (such as llamas, goats, horses and many wildlife species) are susceptible to infection with the Sterne vaccine strain[50]. While humans are quite resistant to infection, it is possible that humans with certain forms of compromised immune competency could be susceptible.

The Pasteur vaccine, which preceded the Sterne vaccine, continues to be produced in a few locations. It is based on an attenuated capsule-forming strain of *B anthracis*.

The usual cattle anthrax vaccine dose is 1 mL and the sheep dose 0.5 mL. The OIE standard states that the vaccine should contain not less than 10 million viable spores per dose for cattle, buffaloes and horses, and not less than 5 million spores per dose for sheep, goats and pigs. However, the Australian Pesticides & Veterinary Medicines Authority and the United States Department of Agriculture[51] accept a minimum of at least 2 million viable spores for cattle and one million for sheep and goats.

The European Pharmacopoeia does not stipulate the minimum spore count in anthrax vaccines but requires that the "number of live spores determined by plate count is not less than 80% of that stated on the label."

Protection is conferred with a single injection with annual boosters recommended for at-risk animals. However, as mentioned above, the actual duration of protective immunity is not known and may be quite variable between animals and species.

In China a live spore suspension of strain A16R is used to vaccinate humans. The vaccine is applied by skin scarification and requires a single dose of 160 – 200 million live spores, followed by a booster 6 to 12 months later. In Russia[52], a vaccine containing a live spore suspension of strain STI-1 (Sanitary Technical Institute) is used. Administration is via skin scarification or subcutaneous injection of a 0.5 mL dose. Two doses are required 21 days apart, with an annual booster.

In the rest of the world, human vaccines do not contain live organisms but substances taken from filtrates of cultures of *Bacillus anthracis*. The Sterne strain is used as the source in the UK and a similar strain in the US. The immunogenic components include protective antigen (PA) and possibly other proteins such as lethal toxin (LT).

PA is non-toxic (unless an active anthrax infection is present) and can be isolated and purified to remove other materials. When used in a purified form in a vaccine, it can induce an immune response which provides protection against anthrax.

The human anthrax vaccine licensed in the United States (Biothrax [previously described as Anthrax Vaccine Adsorbed or AVA]) is produced by the Bioport Corporation (Lansing, Michigan) from sterile filtrates of microaerophilic cultures of an attenuated, unencapsulated nonproteolytic

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[50] Turnbull 1991

[51] 9CFR 113.66

[52] Shlyakhov & Rubinstein 1994

strain (V770-NP1-R) of *B anthracis*, first isolated from a case of bovine anthrax in Florida in 1951[53].

The cell-free culture filtrate, thought to contain predominantly PA, is adsorbed to aluminium hydroxide and the final product contains no more than 2.4mg aluminium hydroxide per 0.5ml dose. Formaldehyde (0.02%) and benzethonium chloride (0.0025%) are present as preservatives[54].

The vaccine is administered subcutaneously in 0.5 mL doses at 0, 2 and 4 weeks followed by boosters at 6, 12 and 18 months. Thereafter annual booster doses are recommended for at-risk individuals. A number of studies suggest greater than 90% production of protective antibodies after the third dose.

BioThrax vaccine is reported to be effective at protecting laboratory animals and cattle from both cutaneous and inhalation challenge with anthrax[55].

A human anthrax vaccine has been produced by the Centre for Applied Microbiology and Research (part of the Department of Health) at Porton Down (UK) since 1956. This vaccine (Anthrax Vaccine Precipitated) is made by precipitating the sterile cell-free culture filtrate of a derivative of Sterne strain $34F_2$ with aluminium potassium sulfate. LF (lethal factor) and EF (oedema factor) are present in this vaccine at levels higher than found in the 1980s in lots of AVA[56].

The UK vaccine contains thimerosol as a preservative and is administered intramuscularly in a regimen of three 0.5ml doses at 0, 3 and 6 weeks with a booster dose 6 months after the third dose and annual boosters thereafter. It is still produced, primarily to protect veterinary and laboratory workers and those employed in "hair and hide" industries such as tanneries, woollen mills and bone-meal factories.

Although the strains used in live human vaccines have residual virulence that results in side effects and are thus not considered suitable for human use in the West, research has shown that the protection induced by a live spore vaccine is greater than for a PA-based vaccine against one of the more virulent (Ames) strains of anthrax.[57]

For their part, killed vaccines are also associated with a relatively high incidence of adverse reactions in humans with one large study finding 5% of vaccinates suffered an adverse reaction[58]. However, most reactions are transient and minor.

In recent years, driven by concerns about vaccine safety and the protracted administration regime, there have been concerted efforts to develop a modern recombinant anthrax vaccine

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[53] Auerbach et al 1955; Puziss et al 1963

[54] Brachman et al 2004

[55] Leppla et al 2002

[56] Turnbull et al 1986, 1988

[57] http://www.mod.uk/issues/gulfwar/info/medical/mcm.htm; Little and Knudson 1986 ; Welkos and Friedlander 1988

[58] Jefferson et al 2005

with a strong safety profile and which provided protective immunity from no more than three doses.

In September 2002 the US National Institute of Allergy and Infectious Diseases began funding the advanced development of a new vaccine based on protective antigen (rPA) produced by recombinant bacteria.

In November 2004 the US Department of Health and Human Services announced that VaxGen Inc had been awarded a contract to produce 75 million doses of the vaccine, sufficient to protect 25 million people, with delivery over three years beginning early 2006[59].

In 2001 the Israel Institute for Biological Research, in cooperation with the Israel Defence Forces and Ministry of Health, announced it had developed a recombinant anthrax vaccine for humans that provides protection with a single dose[60].

There have been other efforts directed towards producing protective antigen using recombinant plants. This has been successfully achieved in tobacco, with one report indicating that sufficient antigen to produce 400 million doses of vaccine could be produced per acre with current knowledge. An 18-fold increase using a commercial cultivar in the field was also achievable, the report suggested[61].

Many similar studies of the application of plant vaccines to animal immunisation are currently being undertaken. Vaccines for six infectious diseases (Hepatitis B, diarrhoea, Norwalk virus, rabies, measles, respiratory syncytial virus) have been successfully expressed in plants and administered orally to animals or humans. Recently, Argentinean researchers reported developing a prototype foot and mouth disease vaccine from transgenic alfalfa plants expressing the FMDV VP1 polyprotein.[62]

The field has attracted the attention of major international companies, with Dow AgroSciences recently signing an agreement with Chlorogen, a research company specialising in chloroplast transfer technology, to develop animal health vaccines from plants.[63]

For human use, the most likely application for this technology would be to produce protective antigen at very low cost. For animals there is also a suggestion that it may be possible to apply the technology to pasture or fodder crops so that livestock are vaccinated as they graze. Alternatively, harvested crops could be incorporated into the feed of feedlot cattle and other livestock.

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[59] Pharmaceutical News 4 November 2004.

[60] http://www.nti.org/e_research/profiles/Israel/Biological/3652.html
Cohen et al 2000

[61] Watson et al (2004) Vaccine 22: 4374-4384

[62] Dus Santosa MJ, Carrilloc C, Ardilab F, Ríosb RD, Franzoneb P, Picconec ME, Wigdorovitza A, Borcac MV (2005). Development of transgenic alfalfa plants containing the foot and mouth disease virus structural polyprotein gene P1 and its utilization as an experimental immunogen. Vaccine 23: 1838- 1843

[63] Animal Pharm, 30 September 2005 p. 6

However, commercially available plant based vaccines are not anticipated in the immediate future[64].

There are also continuing efforts aimed at finding improved and new adjuvants for use with protective antigen (PA), at least one program aimed at finding a simple DNA vaccine (using a plasmid encoding PA) and efforts to use PA as a vehicle to direct extracellular proteins to the cytosol where they could directly stimulate the cell-mediated arm of the immune system[65].

One of the most promising recent developments has been vaccines based on the use of a dominant-negative inhibitory (DNI) mutant to replace PA in PA vaccines. When tested in mice, DNI alone is more immunogenic than PA and elicits significantly higher levels of antibodies against PA. To explain the enhanced immunogenicity of DNI, it has been proposed that the two point mutations in DNI may have improved epitopes of PA allowing better antigen presentation to helper T cells. Alternatively, these mutations may enhance the immunological processing of PA by altering endosomal trafficking of the toxin in antigen-presenting cells. Because DNI has previously been demonstrated to inhibit anthrax toxin, postexposure use of DNI-based vaccines may provide improved immunogenicity and therapeutic activity simultaneously.[66]

4.3 Diagnostic Technology

One of the factors limiting rapid control of anthrax outbreaks is the need to confirm that the disease is actually present.

Suspicions are generally raised when there are carcases of animals that have died suddenly with bloodstained discharges from natural orifices and early putrefaction, particularly in known anthrax areas. However, these signs are not pathognomonic and may also be seen with various other diseases. For example, blackleg, black disease, enterotoxaemia (pulpy kidney), certain plant poisons, arsenic and copper poisoning, pregnancy toxaemia (twin lamb disease) and hypocalcaemia, salmonellosis, snake bite and lightning strike can all produce similar signs[67].

In non-endemic areas, livestock owners and veterinarians would not normally regard anthrax as more than a remote possibility. This appeared to contribute to the delay in diagnosis of anthrax in WA in 1994[68].

A veterinarian in the field or in a feedlot could, if suitably equipped and prepared, perform a microscopic examination of a blood smear from a recently dead animal. If encapsulated bacilli were observed after appropriate staining, this would warrant a presumptive diagnosis and immediate appropriate action while confirmation was awaited from culture and identification of samples.

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[64] Relevant literature identified in Appendix 4

[65] Mi-Li et al 1999; Williamson et al 2002; Ferrari et al 2004; Hermanson et al 2004; Galloway et al 2004; Perkins et al 2005

[66] Aulinger et al 2005

[67] Differential diagnoses of suspected anthrax cases described in the Newsletter of Australia's National Animal Health Information System, Animal Health Surveillance.

[68] Forshaw et al 1996

However, even if a veterinarian is available, they are not typically equipped to undertake this kind of examination. Moreover, bacilli in the blood are not always detectable. A definitive diagnosis relies upon laboratory tests including culture of the organism, requiring from several hours to several days.

Thus in most cases the diagnosis of anthrax is delayed due to the possibility that anthrax is not present, facilities for taking samples are not immediately available or because of the time required by the laboratory to process and report on samples.

However, a rapid anthrax field test kit is being evaluated by DPI Victoria and other groups in collaboration with the US Naval and Medical Research Centre in Maryland. The test kit involves an immunochromatographic test (ICT) that detects the protective antigen of *B anthracis* in body fluids. The kit can be used in the field and provides a rapid diagnosis within 15 minutes.

The ICT has been evaluated on 240 samples from cattle at two knackeries in Victoria and compared with blood smears and culture and was used during a 2004 Victorian outbreak, with culture backup. It was found to have high specificity (98.5 to 100%, 95% CI) under Victorian conditions[69].

The test is said to be highly reliable in fresh cases but is apparently less reliable in decomposing carcases over three days old, giving negative results despite positive cultures.

The test has already been used in the US to determine if individual animal deaths are attributable to anthrax and to encourage livestock owners to take the time to properly dispose of carcases. Similar use is possible in Australia. As the test does not require a veterinarian, it could be used by appropriately trained and skilled abattoir staff to check any animals that die suddenly and by livestock owners on farms to check sudden deaths.

By yielding a result in 15 minutes or sooner a diagnostic test would allow for immediate containment, carcass disposal and area disinfection, thereby limiting the area of contamination and risk to livestock.

It may also be suitable to identify individual animals that are incubating the disease. These animals could be treated with antibiotics while those at risk of infection but asymptomatic or those that were not exposed were could be immediately vaccinated. This may prevent further cases during the period following vaccination when already infected animals contract the disease.

Many other tests are under development with high degrees of sensitivity and specificity for use in diagnosis of anthrax in livestock, for testing for the presence of spores in the environment and for assessing the presence of *B anthracis* in food[70].

[69] JD Muller et al 2004.

[70] Survey of tests described in literature presented in Appendix 4.

5 A Secure Vaccine Supply

Summary

Vaccination is integral to controlling and preventing anthrax outbreaks in the field, although antibiotics, carcase disposal and decontamination are also vitally important.

Since 1998 Australia's anthrax vaccine has been imported from the United States. The same manufacturer is also the sole supplier for the USA and Canada.

Some argue that this is not sufficiently secure. Risks relating to manufacturing interruption, business decisions, transport disruption, regulatory intervention and insufficient supply have been identified.

5.1 Controlling Outbreaks

There are two primary reasons advanced to support the need for a secure supply of anthrax vaccine – controlling outbreaks and preventing outbreaks.

As shown by reactions to the 1997 Tatura outbreak and subsequent smaller incidents, there is a threat to market access from anthrax. Even when an incident is well controlled there is potential for loss of market access, especially if communication with the trading partner is not ideal.

A major outbreak that was not rapidly controlled could be expected to provoke closure of markets, primarily in Asia, perhaps for a prolonged period. An uncontrolled minor outbreak also carries some risk.

Vaccination is generally regarded as integral to controlling anthrax incidents due to its effectiveness and relatively low cost. Within two weeks of a single dose of vaccine, most livestock are likely to be protected from anthrax infection.

Vaccination also reinforces the perception that the outbreak has been contained and reduces the inclination to apply blanket limitations on Australian produce. With appropriate disposal of carcases and decontamination of ground, vaccinated livestock can be quarantined more or less in place.

However, even with rapid and widespread vaccination, cases of anthrax can still emerge for eight to ten days as animals incubating anthrax at the time of vaccination will not be protected immediately. When a diagnosis is made, the farm could already be in the second wave of cases with another wave of animals silently incubating the disease. Vaccination cannot prevent the progression of these cases as deaths typically only stop from the eighth day onwards.

Antibiotics can also play a role in controlling outbreaks and are a first line of control in Britain[71]. In North Dakota and other states of the USA experiencing anthrax outbreaks, the current approach is to treat all potentially exposed stock with a long-acting antibiotic to intercept incubating infections. Vaccination then occurs 7-10 days later when circulating systemic levels of antibiotics have fallen. If studies demonstrate their effectiveness, use of antibiotics with zero or short milk withholding periods (for example ceftiofur) make this an attractive option for consideration in dairy herds.

[71] Turnbull 1998

It is not feasible to administer antibiotics concurrently with the vaccine as the vaccine contains a living organism which will be killed by the antibiotic and prevent the development of immunity[72]. To overcome this significant limitation of living vaccines, use of a vaccine strain that is resistant to antibiotics has been advocated[73].

Dairy cows treated for mastitis with intramammary antibiotics or given dry-cow therapy may have enough antibiotics circulating to affect the vaccine and leave them unprotected from infection after a few days. These animals need to be moved away from the contamination source (eg out of the paddock they were grazing, or on a different feed, whichever applies) until they can be effectively vaccinated and immunity has had time to develop.

In the absence of vaccine, an outbreak could be effectively controlled by administering prophylactic antibiotics to all at-risk livestock. This would not necessarily provide a long-term solution as treated animals return to susceptibility within a few days. Prevention of further cases would require their removal from the source of infection, if known.

Once a new rapid diagnostic test is available, it may be possible to administer antibiotics only to those animals that are confirmed as incubating the disease rather than all exposed stock. That would improve both short and long term prevention of further cases. New detection technology may also improve the likelihood of identifying the sources of infection, permitting decontamination measures to be implemented and decreasing the risks of ongoing exposure.

Given the central role that vaccination plays in any of these scenarios, there is no question that Australia requires an assured supply of effective anthrax vaccine, together with the protocols and resources for using it, to control outbreaks of the disease.

5.2 Preventing Anthrax Incidents

Ideally, anthrax incidents should be prevented from occurring rather than controlled after they occur.

Interest in preventive vaccination is highest in cattle feedlots. Most feedlots targeting Asian markets are now routinely vaccinating cattle against anthrax. It is said that any such feedlot in which an anthrax incident occurred would find it difficult to stay in business.

The most likely origin of anthrax in feedlots is in the introduction of cattle incubating the disease on arrival. Given the rapid incubation period (2-4 days) and the time necessary for protective immunity to develop (7-14 days), the only effective vaccination approach is to ensure that cattle from areas with a high risk of exposure are vaccinated at least two weeks prior to entry into a feedlot.

Other possible but less likely origins of anthrax infection include the feedlot environment itself or via contaminated feed. The feedlot environment, unless situated in an area with a history of anthrax, is generally considered unfavourable to the survival of anthrax spores. Contaminated feed, while historically a major source of anthrax infection, is unlikely under the quality assurance programs operating in the feed industry.

In the past the feedlot industry (via ALFA) has attempted to incorporate anthrax vaccination into its quality assurance protocol, stipulating that cattle must be vaccinated prior to entering a

[72] Lee et al 1961; Webster 1973; Malovastyi 1985

[73] Pomerantsev et al 1996

feedlot. However, with competition for cattle and arguments about who should pay, this has now largely given way to routine vaccination on arrival. Thus the risk of anthrax is little changed and cases could still occur before the vaccine has taken effect.

That risk might be somewhat mitigated if oral antibiotics were administered on entry into the feedlot. Ionophores and virginiamycin have a Gram-positive spectrum of antimicrobial activity and are known to control Streptococcus, Staphylococcus and Clostridia. It is presumed they would also control *B anthracis*. If so, this would provide a barrier to infection via the gastrointestinal tract, the principal route of infection of anthrax in ruminants. The *in vitro* susceptibility of *B anthracis* to streptogramins has been described[74] but susceptibility to the ionophores does not appear to have been reported.

However, routine administration of in-feed antibiotics in feedlots is becoming less common, particularly for those that supply export markets or the domestic supermarkets. Thus this avenue of protection may no longer be acceptable.

In the case of pasture-based cattle and sheep production, vaccination is generally limited to properties on which previous losses have occurred. Even then, there are varying perceptions as to the risk relative to the cost. Anecdotally, it is said that use of the vaccine declines as memories of the last case recede.

A decline in vaccination use following a decrease in anthrax incidence was observed and reported by Henry in 1922[75]. Henry also noted that, "the decrease in the prevalence of disease [anthrax] is due partly to natural causes, and partly to human action. The disappearance of anthrax in many districts was not due to action on the part of man, and no explanation can be offered as to why it ceased to exist. … There is as a rule a tendency to overrate the comparative value of vaccination as opposed to other measures adopted …"

The South African scientist Max Sterne, originator of the current vaccine strain, claimed that from his experience in Natal in the late 1930s, a pasture would stay contaminated for between three months and three years[76].

That observation may specifically relate to initial surface contamination and not to animal anthrax graves, which under exceptional circumstances can remain at risk for centuries.

The policy in Victoria is to provide vaccination for three years following an outbreak, after which animals remain unprotected unless the livestock owner is willing to pay for continued vaccination. This is apparently intended to monitor background infection levels and mirrors the policy adopted in South Africa.

5.3 The Risk To Supply

The ability to rapidly bring an anthrax outbreak under control is a key element in reassuring export markets. Vaccination is a key element of outbreak management plans and assured supply of vaccine therefore underpins Australia's ability to minimise the impact of anthrax.

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[74] Athamna et al 2004c

[75] Henry 1922

[76] Hugh-Jones 1998

If vaccine became unavailable during an outbreak, or if an outbreak resulted from vaccine being unavailable, market access would very likely be adversely affected.

The same is true if a shortage of vaccine occurred during an outbreak, as occurred in 1997 at Tatura. Control would probably take longer and be more difficult to achieve, using antibiotics and shifting livestock from infected ground, with greater potential to alarm export markets. Even domestic markets could be affected if cases kept occurring.

Thus Australia needs an assured supply of anthrax vaccine, including the capacity to rapidly increase supply if an outbreak occurs.

There is currently only one anthrax vaccine registered in Australia, produced in the US by Colorado Serum Company (CSC) [77], and distributed by Fort Dodge Australia. Fort Dodge purchases vaccine from the company on an arms-length basis. There is no long-term supply agreement and Colorado is under no obligation to accept or fill orders.

CSC, established in 1923, is the oldest family-owned veterinary biological manufacturer in the United States. It produces vaccines and anti-sera products for cattle, sheep, goats, pigs and horses, mostly for the US market. It is also the only manufacturer of anthrax vaccine in North America. A full list of the company's products is included in Appendix 6.

CSC is the sole producer of the RB51 *B abortus* vaccine used in the US cattle and bison industries. It developed the first USDA-licensed Bluetongue vaccine in the mid 1960s and has responded to numerous epidemics since the mid 50s, working with Colorado State University to develop vaccines. A separate division purchased in 1959, Western Instrument Company of Chicago, manufactures instruments utilized in the animal and human health fields. The company is a member of the industry organisation, Animal Health Institute[78].

The company also produces products to prevent caseous lymphadenitis, equine encephalomyelitis, Pasteurellosis, tetanus, Clostridial diseases, ovine Campylobacteriosis, Leptospirosis, Brucellosis, Salmonellosis, Papilloma virus, West Nile Virus, Erysipelas, Bluetongue, Contagious Ecthyma (scabby mouth), Ovine Enzootic Abortion (*Chlamydia psittaci),* Bovine Rhinotracheitis, Virus Diarrhoea and Parainfluenza Virus. Several CSC products contain live or killed organisms that are exotic to Australia.

As vaccine companies go, there is nothing to indicate CSC is any more or less likely to be unable to supply vaccine at a critical time.

It has been argued that relying on a single overseas source is not as secure as it ought to be given the significance of the disease as discussed previously. The risk of vaccine unavailability is advanced as a key reason for establishing local vaccine production.

Among the scenarios that could place the supply of vaccine at risk, the most apparent ones are as follows:

Manufacturing Interruption

Major Accident

[77] www.colorado-serum.com

[78] www.ahi.org

- CSC suffers a major disruption to production (eg factory fire) and is unable to manufacture anthrax vaccine for an extended period.

Quality Control Failure

- CSC suffers a manufacturing or quality control problem and is unable to supply vaccine to specification for an extended period.

Seed Failure

- Seed stock unexpectedly loses immunogenicity or becomes contaminated.

Industrial Action

- Strike by manufacturing personnel interrupts supply during time of increased use.

Business Decisions

Entire Business Operation Ceases

- CSC permanently ceases operation due to business failure or owner decision.

Product Rationalisation

- CSC reviews its product range and concludes that anthrax vaccine is not commercially viable. After giving several months notice, it ceases production.

Product Development

- CSC decides to cease production of Sterne vaccine and focus on the recent breakthrough in plant based vaccine (GMO) that is unacceptable to Australian authorities.

Cost Increases

- An upgrade of manufacturing facility and quality of raw materials, combined with increased cost of supplies, necessitates a significant increase in price.

Transport Of Product

- Air and sea freight services involving the shipment of anthrax vaccine between the US and Australia are disrupted for a prolonged period.

Regulatory Intervention

Australian Authorities

- There is an outbreak of exotic disease in the US (such as BSE or FMD) resulting in CSC being unable to satisfy AQIS that its vaccine is free of adventitious pathogens and other agents. As a result, importation of the vaccine is suspended.

- APVMA responds to reports of target animal toxicity or inefficacy, withdrawing approval until additional studies are satisfactorily completed and reviewed.

- Australian government prohibits or restricts import of living *Bacillus anthracis.*

US Authorities

- There is a renewed bio-terrorism threat in the US and the government prohibits the export of anthrax products for “security reasons”.

- A USDA audit reveals adverse reports of toxicity or inefficacy and suspends the product license.

Demand Exceeds Supply

- There is a major anthrax outbreak in Australia for which a substantial quantity of vaccine is required at short notice. However, all CSC production is committed and the additional requirement cannot be supplied in the short term.

- There is a major anthrax outbreak in the US or another country to which CSC supplies vaccine and all vaccine from CSC is required, leaving insufficient (or none at all) for the Australian market.

The scenarios that involve cessation of production by Colorado Serum Company are also relevant to issue of security of supply within the United States and Canada as the company is those countries’ sole source of anthrax vaccine as well.

If the United States or Canada found an acceptable alternative source of anthrax vaccine, there is a good chance it may also be an option for Australia. However, that cannot necessarily be taken for granted. Almost any source inevitably raises quarantine concerns and Australian and US quarantine authorities do not necessarily follow the same approach to assessing risk.

6 Vaccine Demand

Summary

Over the last 10 years, demand for anthrax vaccine has increased from about 250,000 to 400,000 cattle doses per year. The growth is mainly attributable to use by feedlots.

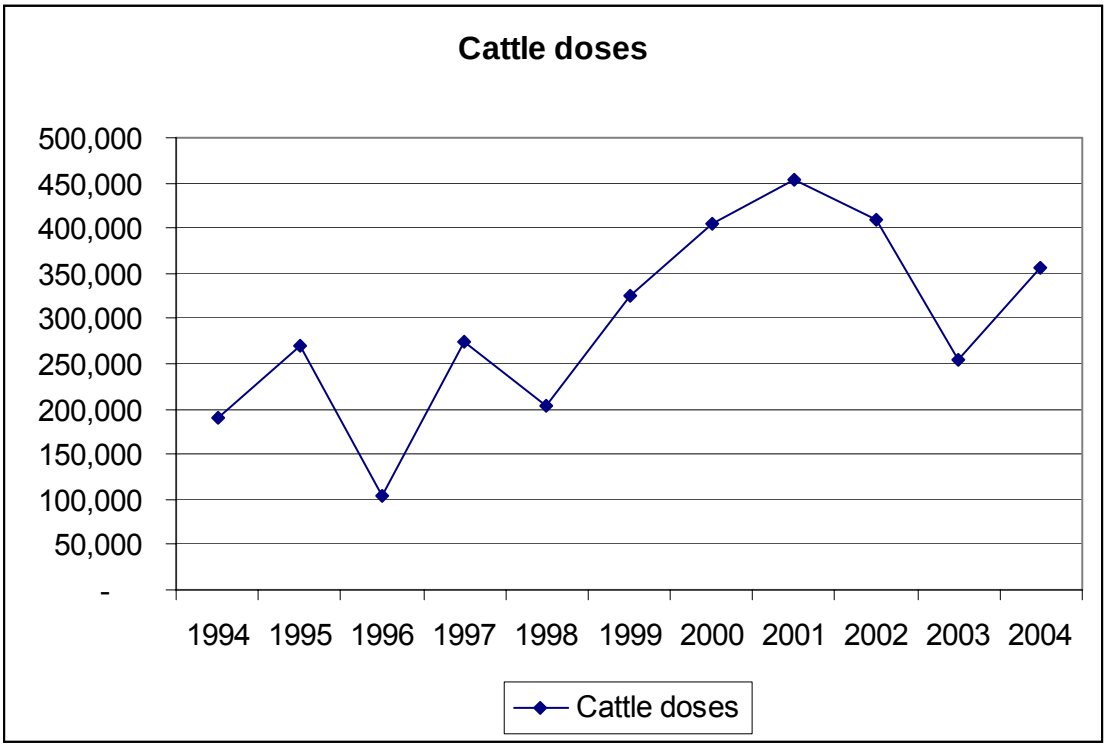
Demand was substantially higher in the mid 1980s due to use in live export sheep but lower in earlier decades. Few grazing livestock are vaccinated except in the face of an imminent threat. Historical reports suggest this may always have been the case.

The Tatura outbreak in 1997 involved the use of 79,000 vaccine doses. This probably represents the upper end of outbreak scenarios.

Terrorist scenarios might amount to similar demand in several locations simultaneously.

6.1 Underlying Demand

Demand for vaccine to control and prevent outbreaks of anthrax in Australia has steadily increased over the last ten years. This is shown by the following graph. Fort Dodges attributes the growth largely to greater preventive vaccination by feedlots.



Demand is likely to continue to rise as more feedlots vaccinate prophylactically in response to market concerns and it may become almost universal. However, fluctuations in the economics of feedlots will also inevitably cause fluctuations in vaccine demand.

Vaccine demand has fluctuated dramatically over the decades and has at times been considerably higher.

Back in the early to mid 1980s there was a very large market for anthrax vaccine for use in live sheep exported to the Middle East. Former Quality Assurance Manager of Arthur Webster Pty

Ltd, Richard Bevan, recalls the company producing two or three 500 litre batches of vaccine a year, representing 1-1.5 million cattle doses or 2-3 million sheep doses, to meet this demand.

It appears that CSL elected to cease anthrax vaccine production soon after the live export market suffered a decline due to problems in Saudi Arabia. Live sheep exports fell sharply in 1989 and 1990.

LiveCorp is not aware of any country currently requiring anthrax vaccination as part of its protocol and considers it unlikely there would be any resumption of demand for anthrax vaccine for use in export sheep or cattle. Rather, most markets require the sheep to originate from anthrax-free properties. A similar requirement (consistent with OIE recommendations) could be applied to feedlots.

Even for dairy cattle exports to China, where anthrax outbreaks are common, the stock must originate from a property that has not suffered an anthrax outbreak in the past two years[79]. There is no requirement for the cattle to be vaccinated.

Routine vaccination of grazing livestock is largely restricted to particularly valuable animals in endemic areas and properties where anthrax has recently occurred. In NSW and Victoria, vaccination of in-contact grazing animals is obligatory in the face of an outbreak and for three years subsequently. In Victoria, the Government pays for both the cost of the vaccine and a veterinarian to administer it.

On unaffected properties, routine vaccination is not encouraged. Kennedy's 1979 study suggests little has changed since that time:[80]

> *"Not surprisingly, preventive vaccination on unaffected properties is employed more frequently in years when there are more incidents of anthrax. …. It appears that stockowners usually wait until there is an outbreak of anthrax infection in their area before they vaccinate their own stock."*

Kennedy suggests that the level of vaccination may have little impact on overall mortality rates of grazing stock considered at risk. He found that over six decades the mortality rates for sheep and cattle remained at between five and 20 deaths per million of each species, notwithstanding fluctuating levels of vaccination.

Kennedy reported that between 1901 and 1905 an average of 1.4 million sheep and 10,000 cattle were vaccinated each year. However, the averages for the period 1916 to 1920 were only 230,000 and 1,080 doses respectively, with no great increase in the number of anthrax incidents reported.

Even when the price of vaccine was substantially lower, as it was in 1979, there was little enthusiasm for routine annual vaccination.

> *"Considering the previously calculated estimates of mortalities [1.9% for cattle and 1.3% for sheep, on average in decade 1965 to 1975]…. when it is considered that few properties would suffer deaths more than once in a decade, the economic benefit of annual vaccination is doubtful, especially for sheep."*

It is therefore anticipated that unless there is a change in the requirements for livestock export, underlying demand for anthrax vaccine will continue to be dominated by cattle feedlots

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[79] LiveCorp pers comm.

[80] Kennedy D. (1979). A study of anthrax in grazing livestock. MVSc Thesis, Faculty of Veterinary Science, University of Melbourne.

supplemented by on-farm vaccination of high value stock and properties where there is known contamination or cases have occurred in recent years.

There is no strong imperative to vaccinate grazing animals except in response to obvious risk and no apparent reason why that should change.

6.2 Outbreak Scenarios

The largest outbreak of anthrax in recent times was in Tatura, north-central Victoria, between January and February 1997[81].

That outbreak involved 83 properties with around 200 cases.

Vaccination was used as a major tool to control the outbreak by establishing a vaccination buffer zone measuring 30 km by 20 km. In all, 78,649 cattle in 457 herds on 83 properties were vaccinated in a three-week program.

As the disease is not spread from animal to animal, investigations into the epidemiology of the outbreak centred on establishing a common source of infection or transmission. The investigators were unable to establish a single major association for the spread of the disease by flies, biting insects, carrion scavengers, wind, manufactured feed, milk factory tanker routes, veterinary visits, animal treatments, movements of personnel between farms or burning of carcases.

The weather conditions in the outbreak area were part of a long dry spell with periods of high day and night temperatures, high humidity and higher than normal soil temperatures.

It is possible that extensive earth works in the district involving irrigated pasture renovation and water channel and drainage renovation could have disturbed old anthrax graves. It is postulated that these works released spores that were dispersed in the preceding wet winter across poorly drained areas that formed the axis for the outbreak.

The axis of the outbreak was the major stock route for cattle and sheep moving from southern Victoria to northern Victoria and southern New South Wales, and undoubtedly there would have been extensive anthrax outbreaks before vaccine became available in the 1890s.

Given the uncertainty regarding the causes of the outbreak, it is not possible to be confident about the potential for a similar outbreak occurring in the future. The potential for such an outbreak is probably no different now from what it was prior to 1997.

Nonetheless, most other outbreaks in Australia both prior to and since 1997 have been far smaller in scale with vaccine requirements significantly less.

OIE reports show 1400 sheep were vaccinated in Australia in 1999, the highest yearly number between 1993 and 2004 associated with an outbreak. That unusual outbreak is reported as causing 400 sheep deaths. Curiously, the reports of anthrax incidents to NAHIS describe the death of only 100 sheep in a mob of 1700[82].

Therefore it would be legitimate to regard the Tatura outbreak as representing the upper limit of any foreseeable scenario involving naturally acquired anthrax in the field. Moreover, while it is

[81] Turner et al 1999a, b.

[82] Sergeant 1999

conceivable there may be simultaneous outbreaks of similar proportions to the Tatura incident, anything outside this would be too far beyond reality to warrant serious consideration.

Moreover, technological improvements in anthrax spore detection now provide an opportunity for pre-emptive environmental assessments of high-risk situations, for example, when large earthmoving projects are undertaken in areas of possible anthrax contamination, as may have been the case in the 1997 Tatura incident.

6.3 Terrorist Scenarios

The potential for anthrax to be used for terrorist purposes against human targets was realised in the US in October 2001[83]. However, except for possible use in World War 1, there are no known examples of anthrax being used as a weapon against animals.

It has nonetheless been suggested that one of the justifications for establishing a more assured supply of anthrax vaccine is to be able to deal with a terrorist release of the disease.

The image has been raised of terrorists scattering virulent anthrax spores from light aircraft over a wide area of the country, perhaps in a coordinated manner across several locations and potentially involving other countries as well. A plan to undertake such a manoeuvre in Germany was considered during World War 2, but never enacted[84].

Another image involves the contamination of livestock feed supplied to feedlots via a major feed mill.

We are not qualified to judge whether the infecting of livestock would serve a terrorist's purposes better than infecting humans, or whether a terrorist would choose anthrax as a livestock disease agent ahead of contagious agents that can be more readily transmitted from animal to animal or animal to human, or even the use of chemicals added to feedstuffs. Contamination of feedstuffs with various organochlorine compounds has a long history in Australia and the impact on trade is well known.

Agencies such as the Australian National Counter-Terrorism Committee[85] are presumably more qualified to judge whether the risk of anthrax as a terrorist weapon is significant.

Agricultural terrorism has been considered in the United States and was recently the subject of testimony to a US Senate committee.[86] That testimony included the following:

> *In 2003, two expert panels were convened by the Federal government to assess the threat from animal pathogens that could be used by bioterrorists or agroterrorists, and to establish research and development needs to reduce the threat from these agents. The Interagency Weapons of Mass Destruction (WMD) Counter Measures Working Group – Animal Pathogens*

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[83] Traeger et al 2002

[84] Bhalla & Warheit (2004

[85] National Counter-Terrorism Plan, National Counter-Terrorism Committee, June 2003 http://nationalsecurity.ag.gov.au

[86] Presented by James A. Roth, Director, Centre for Food Security and Public Health, Iowa State University, College of Veterinary Medicine, Ames, Iowa. Testimony before the United States Senate Committee on Agriculture, Nutrition, And Forestry; July 20, 2005. http://www.cfsph.iastate.edu/News/senate.htm

Research and Development Subgroup (2003) and a White House Office of Science and Technology Policy (OSTP) Agroterrorism Countermeasures Blue Ribbon Panel (Dec 2003), identified 10 animal diseases to be of highest priority for vaccine and anti-viral research and development: foot and mouth disease, Rift Valley fever, highly pathogenic avian influenza, Nipah/Hendra, exotic Newcastle disease, classical swine fever, African swine fever, Venezuelan and eastern equine encephalitis, and rinderpest. The expert groups recommended significant investments in vaccine and anti-viral research and development to mitigate the threat from these agents.

HSPD 9 calls for the creation of a National Veterinary Stockpile (NVS) containing significant amounts of animal vaccine, antiviral or therapeutic products to appropriately respond to the most damaging animal diseases affecting human health and the economy. The NVS should be capable of deploying vaccines within 24 hours of an outbreak. Rift Valley fever (RVF), Nipah virus, and avian influenza are especially significant threats because of their contagious nature and the fact that they can cause serious illness and death in humans. Sufficient data exists to demonstrate that safe and effective vaccines for these three diseases can be developed in a short time frame. A relatively modest investment could result in the development and production of vaccines for these three diseases for the NVS. This preventive measure would effectively reduce the serious threat these diseases pose to both public health and animal agriculture.

If a terrorist scenario involving anthrax was considered sufficiently realistic to warrant contingency planning, one of the consequences may be a substantial increase in potential demand for vaccine. A Tatura-size outbreak of anthrax occurring simultaneously in several states, for example, would increase demand for vaccine by several hundred thousand doses, all potentially required within a relatively short period.

However, an effective response to such terrorism would also require improved diagnostic and detection technology and a range of other measures. Vaccination could conceivably form an important component of a preparedness strategy that ideally would be developed in consultation with those with special expertise in forensic epidemiology and bioterrorism.

6.4 Total Requirements

Australia's total potential need for anthrax vaccine is the sum of its requirements for each source of demand, namely underlying demand plus additional spontaneous or terrorist-initiated outbreaks.

Possible annual totals for each of these are as follows.

Underlying demand	450,000
Tatura scale spontaneous outbreak	80,000
Terrorist outbreak, Tatura scale in three concurrent locations	240,000
Contaminated feed exposes cattle in multiple feedlots simultaneously	400,000

7 Is There Market Failure?

Summary

Within Australia, the market is too small to sustain commercial production. However, internationally the market for anthrax vaccine functions reasonably well and is responsible for the availability of imported vaccine in Australia.

The fact that there is just one vaccine available is mainly a consequence of the regulatory environment.

In economic terms, market failure is a situation in which markets do not efficiently organise production or allocate goods and services to consumers.

The term is normally applied to situations where the inefficiency is particularly dramatic, or when it is suggested that non-market institutions (eg government) would provide a more desirable result.

The two main reasons that markets fail are:

- the inadequate expression of costs or benefits in prices and thus into microeconomic decision-making in markets.
- sub-optimal market structures.

It has been suggested that the anthrax vaccine situation in Australia is an example of market failure. If so, it might also be argued that a more secure supply of anthrax vaccine is unlikely to be achieved in the context of a market response, making a non-market solution necessary.

The anthrax vaccine market in Australia is undeniably small and of limited commercial appeal. The establishment of a local production facility on commercial terms, unless it was also able to supply a significant export market, is unlikely given the capital expenditure required. To that extent, there is market failure.

However, internationally there are quite a few anthrax vaccine producers and a global anthrax vaccine market operates. Moreover, the current importation of anthrax vaccine from Colorado Serum Company by Fort Dodge occurs on commercial terms in response to market demand. Significantly, it also does not appear to be associated with monopoly profits, despite the fact that Fort Dodge is a monopoly supplier. Monopoly profits can be a side effect of market failure.

Efforts to involve Australia in the global anthrax vaccine market on a more extensive scale, by securing a second source of supply, have been thwarted by Australian quarantine barriers.

Thus, while it is true there is market failure if viewed solely as production of vaccine within Australia, on a global basis the market is operating and would operate more effectively but for quarantine policies. It is a moot point whether this amounts to market failure or regulatory failure.

8 A More Affordable Price

Summary

The Industry Working Group has devoted considerable effort to securing anthrax vaccine at a lower price.

Although the current price is substantially higher than it was immediately prior to cessation of local production, it is only about double what it was in 1925 in real terms. There is also no evidence of monopoly pricing by Fort Dodge.

Based on price and demand evidence, there is nothing to substantiate the claim that a lower price for anthrax vaccine would lead to a major increase in demand. However, a promotional campaign focusing on the threat to market access may have that effect.

Options for lowering the price include subsidised production and, potentially, a second vaccine source.

8.1 The Current Situation

It has been suggested that a significantly lower price would lead to a major increase in preventive vaccination and thus reduce the risk of outbreaks further.

Cattle producers that supply feedlots may be less resistant to incurring the cost of vaccination during pre-conditioning, while sheep, beef and dairy graziers may re-evaluate the cost of preventive vaccination relative to the benefit.

Theoretically, the higher the level of routine vaccination in endemic areas, the lower the chances of any further outbreaks in those areas. In practice, a measurable reduction in sporadic incidents could only be expected once vaccination was implemented at a high and sustained rate throughout the anthrax belt. The potential for unprecedented and unpredictable incidents such as those that occurred in WA and Queensland would not change.

This concept appears to have been first raised within the Anthrax Reference Group at its first meeting and has remained one of the priorities of the Industry Working Party.

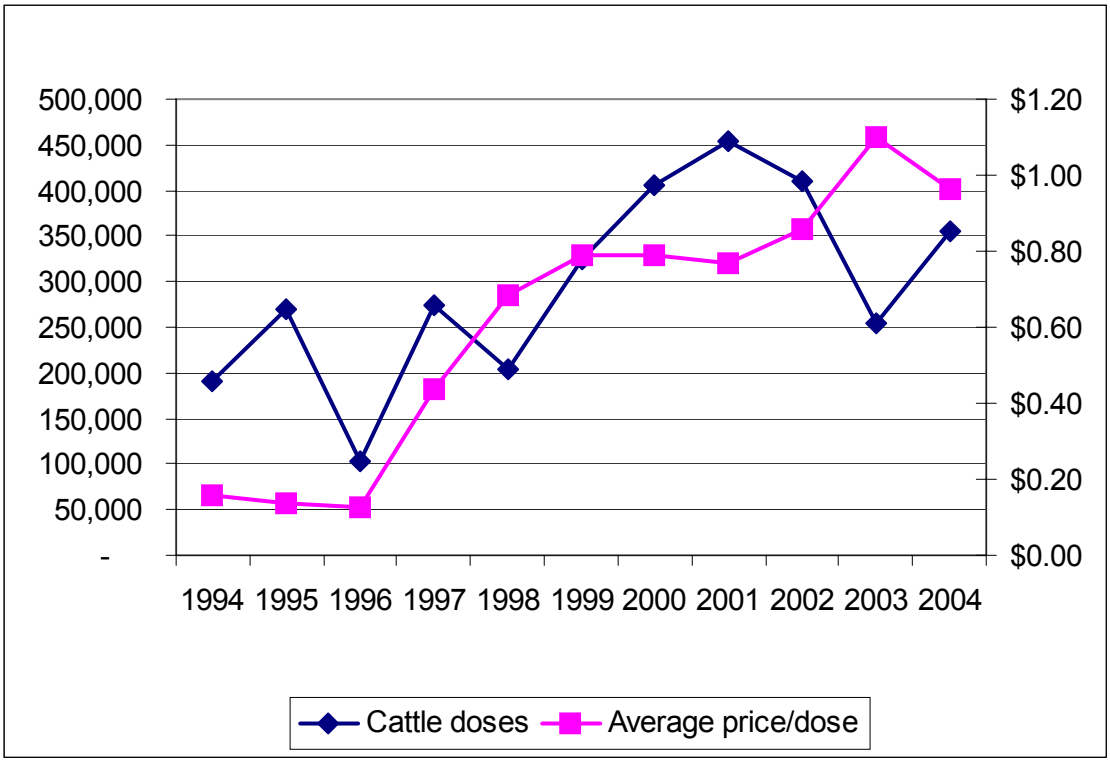
It has even been suggested that if the price of vaccine were to be reduced to very low levels, compulsory vaccination could be justified, aimed at eliminating outbreaks and perhaps eradicating the disease. Against that, the cost of purchasing vaccine is just one of a number of costs incurred in the vaccination of stock.

Based on an analysis of the historical relationship between price and demand, the proposition that a lower price leads to greater use of vaccine (in the absence of compulsion) cannot be supported. Moreover, there is no evidence to show that vaccine demand declined from previously high levels as a result of price increases.

While the price paid by livestock producers for anthrax vaccine has certainly increased considerably since local production ceased in 1997, demand has similarly increased.

Following is a table of sales and price data since 1994, provided by Fort Dodge. Prices are ex-factory and do not include retail margins. The same figures have been graphed, with the price scaled to fit on the graph.

Year	Doses	Price/dose	Revenue
1994	190,500	0.12	22,875
1995	270,450	0.11	29,993
1996	103,450	0.10	10,858
1997	273,600	0.36	98,742
1998	203,500	0.57	116,792
1999	324,350	0.67	216,142
2000	404,800	0.69	278,484
2001	453,400	0.71	322,372
2002	410,250	0.81	333,691
2003	254,250	1.07	272,349
2004	355,900	0.96	343,092



The IWP has suggested that the increase in vaccine demand is attributable to feedlots that, because of their sensitivity to market access risks, have opted to vaccinate prophylactically and are less sensitive to price than graziers. It maintains that, anecdotally, many graziers who previously vaccinated their livestock (particularly sheep) discontinued this because of the increase in price.

We have no data to either verify or contradict that claim beyond confirming that a majority of orders currently received by Fort Dodge is from feedlots. Nonetheless, given the low level of demand for the vaccine, it is obvious that prior to 1997 there must have been very few feedlots vaccinating if graziers were actively vaccinating their stock.

Even if every dose sold at that time had been used in sheep, the total would have been well under 500,000 sheep doses in most years. Moreover, the sharp fluctuations in demand suggest that vaccine use was a response to immediate risk rather than part of a routine program.

In 1922 Max Henry found after examining vaccination records from 1891 to 1920 that graziers tended to vaccinate only when prompted by a recent incident[87].

Kennedy's study in 1979 suggested that preventive vaccination had little impact on anthrax cases the following year[88].

> *"Unfortunately, it appears that preventive vaccination on properties in one year has little if any effect on the severity of disease the following year. There is no inverse relationship between the number of properties vaccinating and the number reporting anthrax in the following year."*

If the price were to fall, it is conceivable that a major promotional campaign incorporating warnings about the risk of market closure could cause a significant increase in routine vaccination among graziers in NSW and Queensland (the cost of vaccination is covered by the Government in Victoria). However, that is speculative and no firm predictions are possible. Amongst dairy farmers in Victoria at the time of the Tatura incident and in the face of milk factory embargoes, there was great reluctance, unless compelled, to vaccinate stock.

Viewed on a longer timescale, the increase in vaccine price is not as dramatic as it first appears. In 1979 the retail price of Webster's anthrax vaccine for a cattle dose was between 8.7 and 12 cents, depending on the pack size[89]. Adjusted for inflation, this would now be 31.3 and 43.2 cents respectively, with ex-factory prices around 28 to 39 cents (and may have been sold at a loss).

According to an advertisement in the first issue of the Australian Veterinary Journal, in June 1925 the cost of the McGarvie Smith vaccine to veterinarians was one penny for a sheep dose and two pence for a cattle dose.

Adjusted for inflation, that represents 59 pence or about 49 cents for the cattle dose. The method of production of the current (Sterne) vaccine is more involved than the McGarvie-Smith product as it contains saponin. In addition, the controls now required for vaccine production add considerably to the complexity and cost. Assuming the McGarvie-Smith Institute was not seeking to generate a profit, this suggests the current price is not drastically higher in real terms than it would have been in 1925 under similar circumstances.

Fort Dodge is sensitive to accusations of unreasonable pricing. It indicated that there were two key reasons for the increase in price of the imported vaccine. These were:

1. The company adopted a policy of ensuring all products it sold generated a profit. Under the prior owners (particularly Arthur Webster Pty Ltd), the vaccine had been sold at breakeven (or in some cases a loss) for many years.

2. The decline in the Australian dollar relative to the United States dollar forced up the cost much more rapidly than would have occurred otherwise.

Notwithstanding the price increases, the product remains a very minor item in the Fort Dodge range and is certainly not regarded as a major source of profit.

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[87] Henry M. (1922). The incidence of anthrax in stock in Australia. J Proc R Soc NSW 56: 44-61

[88] Kennedy D. (1979). A study of anthrax in grazing livestock. MVSc Thesis, Faculty of Veterinary Science, University of Melbourne.

[89] Retail price of Webster's anthrax vaccine on 10 Oct 1979, as quoted by Kennedy (1979) Op cit.

The company aims to achieve a gross margin in the range of 30-40%, although its figures indicate it has actually averaged less than 30% in most years[90]. The company maintains that overheads account for 18% of sales, leaving a net profit of 12% or less at this level.

In the animal health industry generally, gross margins on products in competitive markets are typically in the range of 30-40%. Where there is no competitor product, they are quite often above 50%.

The price paid by livestock producers incorporates a profit margin for rural merchandise retailers or veterinary wholesalers, which sell the vaccine to livestock producers and feedlots. Both of these currently generate a gross margin of about 15%, up from 10% ten years ago. This is additional to the profit made by Fort Dodge.

Whether compulsory vaccination could be justified if the price were very low is beyond the scope of this project. Moreover, the question of whether eradication is actually achievable with universal vaccination would require considerably more investigation.

Assuming it were possible, the price of the vaccine would be a very minor element in an eradication program.

Eradication would also have no impact on the potential for anthrax to be used as a terrorist weapon.

An option for achieving high levels of anthrax vaccination with a low cost product, without resorting to compulsion, would be to incorporate the antigen into an existing and widely used product such as a multivalent Clostridial vaccine. This was the approach used with caseous lymphadenitis (*Corynebacterium pseudotuberculosis*) in sheep and proved to be very effective.

Anthrax vaccines that contain Clostridial antigens are available in South Korea and Uruguay and have been widely described[91].

8.2 Options For Lowering The Price

If it was considered desirable to lower the price of anthrax vaccine to livestock owners, there are two options that could be considered. The second is based on commercial principles, the first is not.

1. Subsidised production

Vaccine production in which the costs of production were not fully recovered would result in a lower price. That is, some of the costs of production would be borne elsewhere.

There are probably only two potential sources of subsidy: livestock owners, through levies; and taxpayers, via the government.

2. A second vaccine source

[90] We were given access to details of gross profit on the vaccine but these are commercially confidential.

[91] Published literature on anthrax combination vaccines is identified in Appendix 4.

A second source of vaccine would tend to put downward pressure on the current price. Although Fort Dodge does not appear to be charging monopoly profits, another vaccine is likely to increase competitive pressure.

This would certainly be the case if the second source was a lower cost supplier or the vaccine was sold in Australia by its manufacturer rather than through a third party. Currently, both Colorado Serum Company as manufacturer and Fort Dodge as distributor make a profit on sales.

There is a risk that if another vaccine were to become available, Fort Dodge would withdraw from the market. This might result in a return to current prices.

The potential for securing a second source of vaccine is discussed elsewhere in this report.

9 Achieving A Secure Supply

Summary

A more secure supply of anthrax vaccine could be achieved by either a second source of supply or a vaccine bank.

By itself, local production would not significantly increase security of supply. While international transport and foreign regulatory risk would be removed, manufacturing and supply risk may actually increase.

A second source of vaccine probably represents the greatest amount of risk reduction for the least cost.

In practical terms, establishing a second source of supply is problematic and a vaccine bank may be the most feasible option in terms of risk reduction relative to cost. There are no domestic vaccine manufacturers willing to produce anthrax vaccine in existing facilities, although Fort Dodge is willing to help establish a vaccine bank.

An international search for a second source of vaccine, beginning in 1998, was not successful largely due to an inability to meet Australian regulatory and quarantine requirements. Nonetheless, there are quite a few manufacturers that were not investigated and circumstances may have changed with others.

9.1 Options and Risks

A more secure supply of anthrax vaccine can be defined as one in which some or all of the current risks to supply do not apply.

That is, a more secure supply would be defined as one that was less exposed to:

- Production risk.
- Business risk.
- Transport risk.
- Regulatory risk
- The risk that supply will be insufficient

This section considers the means by which a more secure supply could be achieved.

9.1.1 A Second Source

A more secure supply of anthrax vaccine could be achieved by simply establishing two acceptable sources rather than just one. The risks previously discussed would be substantially reduced irrespective of the location of the second source or whether its price was higher or lower than at present.

Provided the two sources were separated geographically and financially, the potential for both to be simultaneously affected by any of the scenarios described under the risk to supply is probably too remote to warrant consideration.

If one of the sources became unavailable either permanently or for a prolonged period, there would be a period of increased risk while a replacement source was established.

The initial efforts of the Industry Working Party were mainly directed at establishing a second source. However, if that had led to the availability of vaccine at a significantly lower price, as intended, the second source may well have become the sole supplier. It is likely Fort Dodge (or any similar supplier) would withdraw from the market if another vaccine became available at a substantially lower price. The net effect of this would be no change in security of supply.

The size of the anthrax vaccine market is almost certainly large enough to support two commercial suppliers (based on imported product) provided they both generate a normal margin, have other livestock products to help cover overheads and do not incur abnormal costs such as excessive inventory or stock redundancy. However, approximate price relativity would be required to maintain continuity.

If one of the vaccines were simply registered and available if required, the same reduction in risk would be achieved. That is, it is not essential for both sources to sell vaccine commercially.

9.1.2 This might be the outcome, for example, if Fort Dodge withdrew from the market but registration of its product was maintained. The same outcome could be achieved under several other scenarios, including funding by industry of the registration of a second vaccine. A Vaccine Bank

A vaccine bank is essentially a quantity of vaccine held in storage that is available for use as and when required.

Although a supplier is needed to establish and replenish the bank, supplier failure and the time required to establish a new supplier would be among the risks addressed in determining the size of the bank. For example, if it was considered that a period of two or three years may be required to establish an alternative supplier, the bank would logically carry sufficient vaccine to meet demand for two or three years under whatever demand scenarios were agreed to warrant a response.

Animal Health Australia is proposing to establish a vaccine bank based on one year's normal demand. However, a bank of sufficient size could cope with any demand scenario, irrespective of its plausibility.

The bank could be located in Australia or overseas. The FMD vaccine bank, for example, is located in Europe. If the anthrax vaccine bank was located in Australia, risks associated with a foreign location and international transport would be removed.

Similarly, so long as the vaccine was approved by regulatory and quarantine authorities, the manufacturer could be equally located either in Australia or overseas. Thus it could be comprised of Colorado Serum Company vaccine supplied by Fort Dodge, vaccine produced at a local facility, or vaccine from some other source.

A vaccine bank could be maintained, in whole or part, in the form of frozen antigen ready to be formulated into vaccine. Australia's FMD vaccine bank is held as frozen antigen, with the supplier subject to penalties for failure to formulate and supply vaccine within a specified time.

However, unlike FMD vaccine, formulated anthrax vaccine has quite a long shelf life and a current and ongoing demand for use. Holding frozen antigen may offer no advantage unless the size of the bank was such that formulated vaccine could not be rotated into the market before it became unusable. In that case the retention of some unformulated antigen may be preferable to incurring the cost of disposing of unusable vaccine.

There are additional risks associated with holding frozen antigen rather than formulated vaccine. The formulation facilities may not be available in the event of a crisis requiring a rapid response. There is also a production risk, the main one being potency failure, that would lead to batch rejection. Each of these would prevent vaccine from being available when needed.

9.1.3 Local Vaccine Production

Vaccine production in Australia would avoid the risks associated with international transport and foreign regulatory actions.

However, unless it was additional to the current source or accompanied by a vaccine bank, it would not necessarily increase security of supply in respect of any of the other risks. Some risks may actually increase.

In general terms, a local manufacturer is as susceptible to a factory accident, industrial action, quality control failure or seed-stock failure as a manufacturer located in another country. An industry-owned manufacturer may be protected from business-based decisions but would be vulnerable to management or board decisions having the same outcome.

The individual responsible for managing the WA anthrax incident in the 1990s advised us that vaccine supply from CSL at the time was very unreliable, even with local production. He was told by CSL that anthrax vaccine was very challenging to produce, with two of three batches failing.

Regulatory risk may actually be greater for a local manufacturer compared to a location where GMP reinspections are less frequent.

The risk that demand could exceed supply is also potentially greater for a small-scale local plant. A large plant producing vaccine for several markets would inherently have greater capacity to increase production to deal with a major outbreak.

Further, although the manufacturing process of the Sterne vaccine appears elementary, successful production of concentrated spore bulk antigen is a high-risk process. This was recognised by Sterne and Robinson in the 1930s when they wrote, “More batches are actually started but get discarded at different stages of manufacture.”[92].

More recent manufacturing experience in Australia has confirmed this with reports that half to two-thirds of batches do not attain the minimum spore concentration and must be discarded. Ideally, the manufacturing operation allows optimisation and improvement of the culture conditions and other factors that influence production of successful batches with greater frequency. The literature contains many reports of the importance of culture conditions in spore harvesting as well as reports of improved manufacturing processes, notably the growth of *Bacillus anthracis* in liquid culture[93].

9.1.4 Local Vaccine Production Plus Vaccine Bank

A vaccine bank based on local vaccine production would avoid the risks of international transport and foreign regulatory action faced by a vaccine bank based on an overseas supplier. However, it may face a higher level of manufacturing and local regulatory risk.

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[92] Sterne M, Robinson EM (1939). The preparation of anthrax spore vaccines (for cattle and sheep) in South Africa. Onderstepoort J Vet Sci Anim Ind 12: 9-18

[93] For example, Kiel et al 2000

In reality, assuming banks of sufficient size, both involve quite low risk with no differences of any practical significance.

9.1.5 A Second Source Plus Vaccine Bank

A second source of supply combined with a vaccine bank would probably provide the most secure option of all.

Although the risks associated with a second source of supply or a vaccine bank alone are small, they are not nil. The combination of the two essentially reduces the risk to zero, particularly if the vaccine bank was held within Australia.

9.1.6 The Options Compared

The effect of each option on the risks previously identified is shown in the following table.

	Vaccine Bank	**Second Source**	**Local Production**	**Local Production Plus Vaccine Bank**	**Second Source Plus Vaccine Bank**
Production risk	Largely removed*	Largely removed	No change	Largely removed	Removed
Business risk	Largely removed*	Largely removed	No change	Largely removed	Removed
International transport risk	Removed**	Largely removed	Removed	Removed	Removed
Foreign regulatory risk	Removed	Removed	Removed	Removed	Removed
Risk of inadequate supply	Largely removed*	Largely removed	No change	Largely removed	Removed

* Subject to holding sufficient stock.

** Assumes the vaccine bank is held in Australia.

The cost of each option relative to the reduction in risk is assessed in broad terms as follows:

	Option	**Cost To Establish**	**Risk Reduction**	**Details**
1	Second Source	Low	High	Costs involved in locating a second source probably borne by industry. A second source is assumed to be on commercial terms.
2	Vaccine Bank	Moderate	High	Inventory and storage costs would probably be paid by industry.
3	Local Production	High	Low	Establishment of the facility would be at industry expense.

4	Local Production Plus Vaccine Bank	High	High	Equivalent to vaccine bank. Assumes local production is the only source.
5	Second Source Plus Vaccine Bank	Moderate	Very high	This is the combined effect of 1 and 2.

Based on this, a second source represents the greatest reduction in risk for the least cost.

9.2 Locating A Second Source

In practical terms, a second source of anthrax vaccine for the Australian market could come from any of the following:

- A commercial producer located in another country
- A commercial producer located within Australia or New Zealand (although New Zealand may not be an option as anthrax is now considered exotic to that country.)
- A non-commercial producer located within Australia
- A hybrid commercial – non-commercial producer located within Australia

It is rather unlikely there would be any non-commercial sources located outside Australia.

Whether located overseas or within Australia, any source must be GMP-certified. A source located overseas must also satisfy AQIS requirements to import the vaccine.

9.2.1 Foreign Sources

In 1998 an international search was undertaken by the Commonwealth Department of Agriculture, Fisheries and Forestry for a new source of anthrax vaccine.

According to a letter from Rob Williams (Office of the Chief Veterinary Officer) to Peter Carter dated 16 February 2005, the following anthrax vaccine manufacturers were contacted at the end of 1998.

- Central Veterinary Laboratory, UK
- Institute of Animal Science and Health, Netherlands
- Onderstepoort Veterinary Institute, South Africa
- Colorado Serum Company, USA
- Interifa, Uruguay
- Laboratorio Prondil, Uruguay
- Rhone-Merieux, France
- Phylaxia-Sanofi, Hungary
- Chemo-Sero-Therapeutic Research Institute (CST), Japan
- Choong Ang Animal Disease Laboratory, South Korea
- Bioveta, Czech Republic

The letter indicated that Interifa, CST and Rhone-Merieux did not respond (Rhone-Merieux is now part of Merial) and that CST is a small manufacturer producing vaccine in 10mL vials.

In a further letter dated 17 June 2005, the Department indicated that the Institute of Animal Science and Health in the Netherlands was no longer producing anthrax vaccine.

Subsequent to the Department's inquiries, the Industry Working Party pursued several manufacturers more extensively. Richard Bevan, who has been assisting the Industry Working Party, advised us of the following outcomes:

- Central Veterinary Laboratory Agency (VLA) (UK) produces just 20,000 doses of anthrax vaccine a year and could not provide the quantity required by Australia. Moreover, as a small-scale unit it would not be GMP compliant with a significant increase in volume. [The VLA currently plans to cease production.]

- CZ Veterinaria (Spain) was visited by Richard Bevan and found not to be GMP compliant. The company was willing to upgrade facilities if the cost was covered by Australia.

- Bioveta (Czech Republic) was visited by Richard Bevan and found not to be GMP compliant. The company was also willing to upgrade if the cost was covered.

  At one stage an order was placed with Bioveta but cancelled at the last minute due to an inability to obtain AQIS approval.

- The Onderstepoort plant in South Africa was considered unlikely to meet AQIS requirements.

- Interifa and Laboratorio Prondil (Uruguay) were assumed to be unlikely to meet AQIS requirements and not followed up. (Interifa is part of Rhone-Merieux)

- Choong Ang (South Korea) was assumed to be unlikely to meet AQIS requirements and not followed up.

- Phylaxia-Sanofi has ceased anthrax vaccine production. (Sanofi is now part of Fort Dodge.)

The above list is not exhaustive and there are other anthrax vaccine manufacturers around the world. Moreover, some of the information has changed since that time.

Our internet-based inquiries have confirmed the following organisations appear to produce anthrax vaccine currently.

- Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy[94]

  The company's website (undated) says it produces Pasteur strain vaccine and is about to begin producing Sterne strain. The most recent dated entry is 2002, suggesting the Sterne vaccine may already be available.

- Indian Immunologicals, Hyderabad, India[95]

[94] http://www.fg.izs.it/

In addition to its own products, this plant manufactures Pfizer's vaccines for the Asian market. We understand it has been audited by Pfizer and is considered to be of high standard.

- ChoongAng Vaccine Laboratory Co[96], Daejeon, South Korea

  Produces a combined anthrax-blackleg vaccine.

- Vecol, Bogotá, Colombia[97]
- Central Veterinary Control and Research Institute (Merkez Veteriner Kontrol Ve Araştirma Enstitüsü), Etlik, Ankara, Turkey[98]
- Botswana Vaccine Institute, Gaborone, Botswana (Uses antigen supplied by Merial Uruguay)
- Cooper Kenya, Nairobi, Kenya (Uses antigen from Laboratorio Prondil, Uruguay)
- Pusat Veterinaria Farma, Surabaya, Indonesia
- Merial, Montivideo, Uruguay (formerly known as Interifa)
- All Russian Institute of Veterinary Virology and Microbiology, Vladimir, Russia[99]
- Instituto Rosenbuch, Buenos Aires, Argentina[100]
- San Jorge–Bago, Monte Grande (Buenos Aires), Argentina[101]
- Sanidad Ganadera, Buenos Aires, Argentina[102]
- Biogenesis, Garin, Argentina[103]
- IRFA – Química e Biotecnologia Industrial, Porto Allegre, Brazil[104]

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95 http://www.indimmune.com/

96 http://www.cavac.co.kr/eng/s_cp_01.htm

97 http://www.vecol.com.co/home.htm

98 http://www.etlikvet.gov.tr/MrkVet-Eng2/mainpage.htm

99 http://presentvo.avo.ru/english/3economics/manufactures/14_vniivm/vniivm.html

100 http://www.rosenbusch.com.ar/argentina/home.htm

101 http://www.sanjorge.com.ar/index_eng.html

102 http://www.isg.com.ar/

103 http://www.biogenesis.com.ar/ingles/home.htm

104 http://www.irfa.com.br/

- Laboratório Hertape, Juatuba, Brazil[105]
- Vallée SA, São Paulo, Brazil[106]
- Labovet Produtos Veterinários, Feira de Santana, Brazil [107]
- Laboratório Leivas Leite, CEP, Brazil[108]

WHO[109] also lists the following as anthrax vaccine manufacturers. We could not confirm this by online search.

- Laboratório Vencofarma do Brasil Ltda, Londrina, Brazil[110]
- Laboratório Agromédica, Contagem, Brazil
- Merial, Buenos Aires, Argentina
- Agreed Laboratorios, Buenos Aires, Argentina[111]
- Immunovet, Buenos Aires, Argentina
- Calier, Barcelona, Spain[112] [No anthrax vaccine shown on website]
- Veterina Ltd, Kalinovic, Croatia[113] [No anthrax vaccine shown on website]
- Ovejero, Leon, Spain[114] [No anthrax vaccine shown on website]
- Iven, S.A. Laboratorios e Industrias, Madrid, Spain
- Veterquímica, Santiago, Chile
- Animal Husbandry Laboratory, Dhaka and Cossilla, Bangladesh

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[105] http://www.hertape.com.br/

[106] http://200.219.239.3/vallee/index.php

[107] http://www.labovet.com.br/

[108] http://www.leivasleite.com.br/

[109] WHO 2006

[110] http://www.vencofarma.com.br/

[111] http://redcame.org.ar/ofertaexportable/ver_empresa.php3?id=11

[112] http://www.calier.es/eng/index.html

[113] http://www.veterina.hr/

[114] http://www.labovejero.com/web800/i_default.asp

- National Control Institute of Veterinary Bioproducts & Pharmaceuticals, Beijing, China
- National Veterinary Institute, Bebre, Ethiopia
- Biological Products Division, Directorate of Animal Health, Kathmandu, Nepal
- Vaccine Production Laboratories, Quetta, Pakistan
- Institutul National de Medicina Veterinara "Pasteur", Bucharest, Romania
- Central Veterinary Research Institute, Lusaka, Zambia
- Lanavet, Garoua, Cameroon
- Central Veterinary Laboratory, Tamale, Ghana
- Km 2 Route de Casablanca, Rabat, Morocco
- National Veterinary Research Institute (INIVE), Maputo, Mozambique
- Livestock Breeding and Veterinary Dept, Mukteswar, Myanmar
- Laboratoire Centrale de l'Elevage, Niamey, Niger
- Animal Health Directorate, Syria
- Animal Health Directorate, Ha Tay, Vietnam

Most of these are unlikely to be able to satisfy APVMA and AQIS requirements although several (the Italian, Indian, Argentinean and South Korean sources, for example), could not be ruled out.

In recent years there has been a general upgrading of vaccine production standards in many countries as a result of international trade requirements. This may have increased the potential for some plants to meet Australian requirements, including in locations previously discounted by the IWP.

The resources (time and manpower) involved in contacting each site for preliminary assessment are not great. Moreover, the UK VLA is seeking a source of anthrax vaccine which, if found, may also meet the requirements of AQIS and APVMA.

New biological products from foreign manufacturers are regularly being approved for use in Australia by AQIS and APVMA.

It is both plausible and possible that a foreign anthrax vaccine may be identified. However, we were unable to confirm the potential for any of the listed sources to supply Australia as we were instructed by Meat and Livestock Australia, on advice from the Industry Working Party, that this was not required as part of the project.

9.2.2 Domestic Sources

There are currently eight veterinary vaccine manufacturers in Australia and one in New Zealand. These are:

Allied Biotechnology	Sydney operations Small scale autogenous bacterial vaccine production

Bioproperties	Sydney operations Produces bacterial and viral vaccines for poultry
Fort Dodge	Sydney operations Produces bacterial vaccines for livestock, poultry and pets.
Intervet	Bendigo (Vic) and NZ operations Produces bacterial and viral vaccines for livestock, poultry and pets.
Pfizer (formerly CSL)	Melbourne and NZ operations Produces bacterial and viral vaccines for livestock, horses, poultry and pets.
Progen Industries	Contract manufacturer of bacterial and viral vaccines plus recombinant proteins
Schering-Plough	New Zealand operations Produces bacterial vaccines for livestock.
Tick Fever Research Centre (Qld DPI)	Brisbane operations. Produces tick fever (*B bovis*) vaccine only.
Numico Research	Adelaide viral vaccine manufacturer.

Most of these have been contacted by the IWP at various times over the past seven years.

Some have indicated they would be willing to consider producing anthrax vaccine were it not for the cost of establishing a separate production facility. Primarily for GMP reasons, they are reluctant to manufacture the vaccine in facilities in which other products are produced.

This attitude was documented on two occasions. In 1998 when CSL advised it would not be resuming vaccine production, the company indicated that unless the cost of a new facility was underwritten by industry it would be obliged to charge a similar price to that of the imported vaccine.

In a letter to the IWP dated 17 July 2001, Allied Biotechnology agreed to manufacture and sell vaccine at 52 cents per cattle dose, to meet the Working Party's price expectations, but sought industry funding of a manufacturing plant that it could ultimately acquire.

Our investigations were consistent with the Working Party's experience.

Bioproperties (David Tinworth) said that a separate facility for producing anthrax vaccine was the only way it could be GMP compliant. He believes Akabane Disease vaccine, which his company is considering producing, could not be produced in the same facility as anthrax vaccine. He says that even producing anthrax vaccine on the same site as other vaccines would raise difficulties with export markets.

Allied Biotechnology (Bob Johnston and Mark White) confirmed that their company could not produce anthrax vaccine in its current facilities and had reservations about the attractiveness of the market. It may, however, contemplate producing vaccine at a facility at EMAI.

Fort Dodge, Pfizer and Intervet have large-scale facilities that are not suitable for anthrax production. They are also aware of the anthrax vaccine market and have no interest in producing vaccine for it, other than perhaps on a subsidised basis.

Schering-Plough is not familiar with the anthrax vaccine market. Nonetheless, the company (Ian Pawson) indicated that its plant in New Zealand was designed for large-scale manufacturing, fermenting antigens in 4,000 litre batches with vaccine blends up to 6,000 litres per batch. Its facilities are not designed for producing a product with less than 500 litres a year. Thus there is no possibility of anthrax vaccine manufacture even if NZ biosecurity permitted it.

Numico Research, based in Adelaide, produces mainly viral vaccines plus small quantities of bacterial vaccine. It is not interested in the anthrax market and in any case would be very wary about becoming involved with a spore-forming organism.

Progen (Greg Orders), based in Brisbane, is a toll manufacturer of vaccines. The company does not have the facilities to handle anthrax and also believes it would not be cost competitive.

10 The EMAI Option

Summary

The EMAI option envisages construction of a vaccine production facility on land provided rent-free by NSW DPI at EMAI. Industry would fund establishment while the vaccine would be sold at cost, not including cost of capital.

It is assumed the Sterne strain vaccine would be produced with a minimum spore count of $10x10^6$ spores/ml and that PC3 level biological containment would be provided. A multi-purpose facility is an option if designed accordingly.

The EMAI site appears suitable with the possible exception of the animal house. However, this may not be relevant as batch testing in guinea pigs appears to be unnecessary.

The vaccine will require registration with the APVMA. Overall, there do not appear to be any major hurdles to achieving this once the manufacturing stages are successfully completed.

While the technology of production of the Sterne anthrax vaccine has not changed since 1937, the environment in which the vaccine is manufactured bears little resemblance. The key requirement is to conform to the APVMA's code of Good Manufacturing Practice. This is currently being revised, placing even greater emphasis on Quality Assurance and the production of a reliable and reproducible product.

Up to six personnel are required. In order of increasing feasibility the options include the use of EMAI staff, experienced external staff, or involvement of a third party vaccine manufacturer. In each case, experienced manufacturing and quality assurance managers would be essential.

Three potential sources of master seed organism are available.

A proprietary company limited by shares would meet the entity requirements of anticipated participants while also offering flexibility for the future. A board of three to six directors would be sufficient, representing shareholders and other interests.

Product liability insurance is obtainable, albeit at substantial cost. Alternatives include self-insurance, indemnity by a third party, and manufacture and sale by a third party which already has insurance.

A system for receiving and despatching orders, managing funds and responding to complaints would be required. Ordering via a consortium member such as ALFA or NSW Farmers is an option, with distribution via rural merchandise retailers. Beyond advising how to order vaccine, there is no necessity for marketing.

10.1 General Requirements

In broad terms, the elements required to produce anthrax vaccine in Australia are described in Appendix 2 of the report by Dr Peter Claxton dated 12 June 2002[115] and the FAO Manual for the Production of Anthrax and Blackleg Vaccines[116] and include:

1. A manufacturing facility licensed by the APVMA to produce live bacterial vaccines
    a. Secure freezer storage (<-20°C) for master seed bacteria and prepared antigen.
    b. General storage for raw materials and consumables until required.
    c. A place to culture and harvest the organism
    d. An area to formulate the diluted and adjuvanted vaccine.
    e. A separate room to fill and package the vaccine.
    f. Cold storage for finished goods.
    g. A laboratory equipped for bacteriological work.
2. Appropriately skilled production and quality assurance personnel.
3. Premises for housing guinea pigs for potency testing using a virulent challenge strain of *Bacillus anthracis*.
4. Premises for housing sheep for batch safety testing the vaccine.

To ensure that vaccine strains and challenge strains of *Bacillus anthracis* are kept separate with no possibility of cross-contamination, potency testing should be undertaken on separate premises.

At present the vaccine production facility must meet PC3 requirements for containment as *B anthracis* is listed in Risk Group 3[117]. However, the Australian standard for safety in laboratories is currently being revised with public consultation expected later in 2005.

If the new standard differentiates between virulent *B anthracis* strains and those that have been permanently attenuated such as the Sterne vaccine strain, then the manufacturing facility may only need to meet the conditions of PC2. The conditions of PC3 include those of PC1 and PC2 with additional requirements addressing laboratory facilities, laboratory ventilation, personal protective clothing and equipment, containment equipment, work practices and health monitoring.

The animal house for vaccine potency testing with guinea pigs will almost certainly continue to require PC3 level containment as it involves the use of a strain of *B anthracis* that is virulent to guinea pigs, though reported to be less virulent for humans.

In addition to biological containment of *B anthracis*, the issue of biosecurity is also under review by the Australian Attorney General's Department with a report and recommendations expected later in 2005. Although at least one submission to this review has proposed that only weaponised *B anthracis* attract the highest level of security, the final recommendations are

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[115] "Review of Proposal to Produce Live Anthrax Vaccine at the Elizabeth Macarthur Agricultural institute Menangle, NSW 2568" Report prepared for NSW Agriculture by Dr Peter Claxton, 12 June 2002

[116] Misra (1991). (Appendix 5)

[117] Physical Containment level; AS/NZS 2243.3:2002: Safety in laboratories – Part 3: Microbiological aspects and containment facilities. Standards Australia, Sydney; Standards New Zealand, Wellington

currently not available. If stringent security requirements are recommended then it is expected that additional costs will be incurred in meeting the standard.

All elements of the facility must comply with the Australian Pesticides and Veterinary Medicines Authority (APVMA) code of Good Manufacturing Practice.

The vaccine must be registered with the APVMA. As there is already a registered anthrax vaccine available, selling the product under permit is not an option[118].

It is assumed the anthrax vaccine will use the Sterne strain of *Bacillus anthracis*. Having been manufactured and sold in Australia for decades, such a vaccine would be the easiest to register and is probably the only practical and immediate option.

10.2 Spore Count

The IWP has asked for the assessment of local manufacture to be based on a vaccine containing at least ten million spores per millilitre on the basis that this level is required to guarantee twelve months immunity. The OIE[119], FAO[120] and WHO[121] recommend that anthrax vaccine should not contain less than 10 million culturable spores per dose for cattle. The BP(Vet)[122] and European Pharmacopoeia[123] do not specify a minimum spore count, but require that "the number of live spores determined by plate count is not less than 80 per cent of that stated on the label".

The USDA[124] requires that each batch must have "a spore count sufficiently greater than that of the vaccine used in the immunogenicity test to assure that when tested at any time within the expiration period, each serial and subserial shall have a spore count of at least twice that used in the immunogenicity test but not less than 2,000,000 spores per dose." The current vaccine approved by the APVMA and sold in Australia is manufactured in the US and according to the label contains "not less than $4x10^6$/ml of living spores of *Bacillus anthracis*".

Peter Carter has undertaken a literature review to support the argument that the current vaccine may not provide 12 months protection. Although in contrast to the opinion of OIE[125], this view corresponds with that expressed by Martin Hugh-Jones (Professor Emeritus, Environmental Studies Department, School of Coast & Environment, Louisiana State University, USA) who has observed in field situations that the Colorado Serum Company vaccine provides six to nine months effective protection in cattle.

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[118] "Permits are not intended to be used to circumvent the normal process of registering products and approving the uses on their labels. Therefore, permits will not be granted for an off-label use (OLP), for an emergency use (EP) or to allow supply/use of an unregistered product (SUP), if there is a registered product currently available for that purpose." http://www.apvma.gov.au/minor_use/general.shtml#Role%20and%20types%20of%20permits

[119] OIE (2004). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.2.1. Anthrax.

[120] Misra RP (1991). (Appendix 5)

[121] WHO (1967). Requirements for Anthrax Spore Vaccine (Live, for Veterinary use) (Requirements for biological substances No. 13) WHO Expert Committee on Biological Standardization. Nineteenth Report. (WHO Technical Report Series, No. 361, 1967), pp .31-40

[122] British Pharmacopoeia (Veterinary) (2004). Anthrax vaccine, living. Page 160.

[123] European Pharmacopoeia (1997). Anthrax spore vaccine (live) for veterinary use. 1997:0441, 399-400

[124] APHIS, USDA (2004). 113.66 Anthrax spore vaccine – non-encapsulated. 9CFR Ch. 1 (1-1-04), pp 622-623

[125] OIE (2004) Op cit

In reality, the duration of immunity in cattle may not be directly related to spore count alone. In 1979, when locally produced vaccine invariably contained at least ten million spores per millilitre, Kennedy[126] claimed that it provided less than a year's protection in cattle but three or four years protection in sheep. This view was similarly based on a review of the literature supported by the opinions and field observations of "senior field veterinarians". It should also be recalled that, as mentioned previously, there are a number of reports from Australia of vaccinated cattle succumbing to anthrax from two weeks to eight months after vaccination.

Although it sounds plausible, the hypothesis that increasing the spore concentration from four million to ten million per ml will provide 12 months protection has not been the subject of a published investigation. Without such data, a label claim for 12 months protection would not be possible.

Depending on epidemiologic factors (such as the duration, frequency and magnitude of exposure to anthrax spores), 12 months protection may or may not be necessary to provide effective anthrax control, particularly in feedlots when exposure is most likely prior to arrival and ongoing exposure to anthrax infection is highly unlikely.

10.3 The EMAI Proposal

The Industry Working Party has obtained the agreement of NSW Department of Primary Industries to establish a facility to manufacture vaccine within the grounds of the Elizabeth McArthur Agricultural Institute (EMAI).

This, together with the use of EMAI staff to manufacture the vaccine, is the IWP's preferred option.

The expectation of the IWP is that the capital cost would be shared among industry bodies in a comparable manner to the Emergency Animal Disease Preparedness cost sharing agreement, while the vaccine would be sold "at cost" (i.e. cost of production) to livestock producers. The IWP anticipates this cost would be substantially less than the current price.

The proposal is based on the premise that no existing vaccine manufacturer is willing to undertake anthrax vaccine production without significant underwriting of the capital costs of establishing a plant.

The IWP is reluctant to recommend the commitment of such capital by industry bodies if a private company were to own the asset. It is especially opposed to the idea of providing capital to a private company located in another country. Its concerns relate primarily to whether a private company could or would ensure continuity of supply and whether contributed funds would be secure.

The terms of the Department's offer are set out in letters from the NSW Minister for Primary Industries to the Chairman of the IWP, Peter Carter. They are summarised as follows:

1. Land would be provided to the 'consortium'[127] at no cost. [This effectively means free of rental costs.]
2. All other costs and liabilities would be met by the consortium.
3. If EMAI staff are utilised, this would be "at cost" to the consortium. This is defined as salaries plus on-costs for the period allocated to the project.

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[126] Kennedy D. (1979). Op cit.

[127] Members of the 'consortium' are understood to comprise Cattle Council of Australia, Australian Lot Feeders Association, NSW Farmers Association and State Council of Rural Lands Protection Boards.

4. Tenure would be provided via contract for a period of ten years with a renewal option.
5. The facility must be built to PC3 standard.
6. Similar security would be required as is currently used at EMAI.
7. NSW DPI would seek approval for the building project and be responsible for its construction on behalf of the consortium. The consortium would be responsible for all costs.
8. If a multi-functional facility were built, the cost of decontamination between producing anthrax vaccine and other products sought by NSW DPI would not be borne by the consortium.
9. NSW DPI would seek to be represented on the board of management of the consortium.
10. NSW DPI envisages the location of the facility would not be far from existing EMAI buildings, to aid overall management and allow greater interactions with staff.
11. If EMAI personnel are to be used for production, reasonable notice will be required to ensure they are available when required.

Subsequent to submission of this report in draft form, the IWP advised the consultants as follows:

> *The IWP has for 8 years realised the consequences of running out of vaccine. Back in 1998/99 when importation was progressing with the Czech Republic a vaccine bank of 200,000 was proposed due to the 3-4 months delay in supply after ordering. This was shelved when the deal fell through.*
> *The IWP has always planned to store one years' supply of vaccine in ready-to-use form and a supply of 'spore concentrate' which can be used for decades without deterioration and can be made up into vaccine within a week. Both these resources were always on the drawing board and are intended to safeguard the supply of vaccine under any emergency. The spore concentrate would not necessarily all be stored at EMAI.*

Neither Claxton's two reports nor the correspondence referred to above contains any reference to a vaccine bank in conjunction with production at EMAI.

In discussions with the IWP there was consideration given to the need to carry sufficient inventory to satisfy demand between production campaigns, an undefined period that could potentially be up to 12 months. This is an obvious requirement. However, this was not interpreted as equivalent to a vaccine bank. Indeed, when a vaccine bank was discussed as an alternative to local production, the IWP's view was that such a bank would need to contain several million doses, far more than 200,000 doses or a year's supply as mentioned here.

Nonetheless, on the assumption the EMAI proposal incorporates the creation of a vaccine bank of a year's supply, the impact of this on risk reduction has been assessed in the previous section. The cost impact, from inventory and storage, is included under establishment costs.

10.4 Scale Of Facility

There are two general options in respect of the scale of a facility that could be established at EMAI.

1. A dedicated anthrax vaccine production unit. This would only need to be quite small, to reflect the volume of vaccine required.
2. A multi-purpose unit in which anthrax vaccine may be one of several biological products produced. This would be somewhat larger.

The IWP has expressed a preference for the latter option as it envisages the plant being used to produce vaccines for diseases that are not prevalent enough in Australia or globally to warrant commercial production.

That would necessitate a source of funding for the production of such vaccines and raises a range of issues that are beyond the scope of this project.

A multi-purpose plant may be of interest to local commercial vaccine manufacturers that require manufacturing capacity for a limited period. There is precedent for this in Australia. If such multiple uses were required, it would be necessary to take any likely needs into consideration during the design stage.

10.5 EMAI Suitability

There are several sites at EMAI that appear suitable for erecting a vaccine production facility. All are within close proximity to existing buildings so that services such as electricity and steam could be provided.

The area is subject to subsidence due to underground mining. As a result, the building code requires building slabs to be more heavily reinforced and services to be provided overhead rather than below ground. This increases the cost of construction but is not a barrier to the project itself.

The electricity supply to the site is currently insufficient to accommodate extra demand. The supplier (Integral Energy) has indicated it will only upgrade if there is a development leading to a significant increase in demand.

EMAI is in the process of implementing new building security systems and procedures including card access control, movement detectors and video monitoring. A back to base alarm system is in place. There is no permanent security guard on the site although a security contractor visits during the night. A need for upgraded security may be one result of the Attorney General's review referred to above.

In the opinion of EMAI staff, a section of one of the existing animal houses is capable of being upgraded to PC3 level to accommodate guinea pigs for batch efficacy testing. Guinea pigs can be purchased for the purpose and there would be no need to maintain a permanent colony.

However, it is anticipated the required upgrading would be fairly extensive. In 1998 Dr Peter Claxton reviewed EMAI facilities for the purpose of anthrax vaccine work. In his opinion the animal rooms "were not designed to handle the organism concerned" and "not capable of achieving the level of containment required"[128].

EMAI is an approved quarantine facility and could, if required, import and hold virulent *B anthracis* for efficacy testing subject to satisfying physical containment and security requirements.

EMAI personnel are not familiar with live bacterial vaccine production. Moreover, virtually all of them are funded on a project basis. Notwithstanding the "part-time" requirement, it is uncertain whether they could be released from other activities to produce vaccine each time they were required. The Director of EMAI expressed some reservations about this.

10.6 Regulatory Requirements

A diversity of Commonwealth, State and local regulatory provisions apply to the manufacture and sale of a living anthrax vaccine for livestock.

[128] Anthrax Vaccine Proposal. Evaluation of Facilities at EMAI, Camden. Claxton Consulting, 24 August 1998.

At the Commonwealth level, an anthrax vaccine produced at EMAI will require registration with the APVMA. The APVMA (under Section 14 of the Agricultural and Veterinary Chemicals Code Act, 1994) must be satisfied that:

- the product would not be an undue hazard to the safety of people exposed to it during its handling or people using anything containing its residues;
- the product would not be likely to have an effect that is harmful to human beings;
- the product would not be likely to have an effect that is harmful to animals, plants or things or to the environment;
- the product would not unduly prejudice trade or commerce between Australia and places outside of Australia;
- the product would be effective according to criteria that the APVMA has proposed for the product;
- that any requirements prescribed by the regulations have been complied with.

Submissions for registration by the APVMA need to address issues of manufacturing, efficacy, safety, environment toxicity, occupational health and safety, residues and quarantine. [129]

Efficacy

Discussions with the APVMA as part of this project indicated that specific efficacy studies in sheep or cattle are not necessary in view of the long history of use of the Sterne vaccine.

However, a convincing argument (based on a review of the relevant literature and any other information) would need to be provided as part of the regulatory submission in support of the proposed vaccine. The literature identified in Appendix 4 could be expected to comfortably address this need.

Target Animal Safety

Each batch of vaccine would need to be assessed for safety in the most sensitive target species. Safety studies described by 9 CFR (the US standard), the European Pharmacopoeia and the British Pharmacopoeia (Veterinary) require the administration of twice the dose stated on the label to two sheep of the minimum age for which the product is recommended and previously unexposed to anthrax followed by daily observations of systemic and local responses for 14 (BP(Vet) and EP) or 21 (21 CFR) days.

Potency / Immunogenicity

Compliance with the relevant requirements of 9 CFR, European Pharmacopoeia or the British Pharmacopoeia (Veterinary) is expected to satisfy the needs for potency or immunogenicity testing. However, there is considerable difference between these reference documents.

The monographs for ANTHRAX SPORE VACCINE (LIVE) of the European Pharmacopoeia (1997: 0441) and the British Pharmacopoeia (Veterinary) (2004) describe a potency test in guinea pigs, rabbits or sheep, using test animals vaccinated with 1/10 of the smallest dose on the label for sheep and challenged 21 days after vaccination with 100 MLD (median lethal doses) of a strain of *Bacillus anthracis* pathogenic for the test species. The potency test is applicable to

[129] Guidelines for Format and Data Requirements for Applications to Register New Veterinary Immunobiologicals in Australia. http://www.apvma.gov.au/guidelines/vaccine.shtml

each batch of vaccine and all vaccinated animals must survive challenge while all unvaccinated controls must die in order for the batch to be released.

Because the potency test uses a strain of *Bacillus anthracis* that is pathogenic to the test animal species it must be undertaken in a facility with appropriate physical containment and security. Before commencing the study it is necessary to have determined the MLD for the pathogenic strain of *B anthracis* in the strain of test animals (usually guinea pigs). We have been informed by the Head of Scientific Services Unit at the UK Veterinary Laboratories Agency that the potency test is very demanding and many vaccine batches fail the initial test (a false positive result) though on the basis of viable spore count they would have been expected to protect vaccinated livestock under conditions of field exposure.

The British Pharmacopoeia (Veterinary) (2004) monograph on Veterinary Vaccines discusses the purposes of tests of potency and immunogenicity[130] and states that, "for most vaccines, the tests cited under Potency or Immunogenicity are not suitable for the routine testing of batches." The monograph further states that, "for live vaccines, the minimum acceptable … bacterial count that gives satisfactory results in the Potency test and other efficacy studies is established during development. For routine testing it must be demonstrated for each batch that the titre or count at release is such that at the end of the period of stability, in the light of the stability studies, the vaccine, stored in the recommended conditions, will contain not less than the minimum … bacterial count determined during development studies."

Finally, in clarifying the descriptions of potency tests in specific vaccine monographs, the general monograph on Veterinary Vaccines concludes, "where a batch potency test is described in a monograph, this is given as an example of a test that is considered suitable, after establishment of correlation with the potency test …". A reasonable interpretation of this discussion in the BP(Vet)(2004) is that it should be possible to establish a correlation between live spore count and *in vivo* potency testing during the development of the new vaccine.

The US Code of Federal Regulations Title 9 Part 113.66 describes the standard requirements for Anthrax Spore Vaccine – Non-encapsulated. The standard includes a requirement that each lot of master seed be tested for immunogenicity in a vaccine challenge study using guinea pigs (30 vaccinates and 12 controls). Test animals are challenged 14 to 15 days after vaccination of test animals with not less than 4,500 guinea pig LD50 (equivalent to MLD) of a virulent suspension of *Bacillus anthracis*. Test animals are observed for 10 days and an acceptable vaccine test results when 10 of 12 unvaccinated control animals die while at least 27 of 30 vaccinated animals survive. The code further requires that the master seed, if still in use, is re-evaluated every 3 years. For release of individual batches made from the master seed, final container samples must contain a viable spore count of at least twice that used in the immunogenicity test but not less than 2 million spores per dose.

The vaccine currently available in Australia is manufactured in the US where it is subject to the requirements of 9CFR 113.66. A similar vaccine manufactured in Australia can be expected to require a standard of potency no greater than that currently accepted.

It is therefore concluded that batch release will not invariably require potency testing if a relationship between spore count and potency can be established. Potency testing of the master seed plus testing of the batches used to support stability should enable the relationship between spore count and potency to be established. If this relationship is accepted by APVMA, further batches could be released on spore count with reassessment of the master seed every three years.

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[130] BP(Vet)(2004). Veterinary Vaccines: Potency and immunogencity, page 157

Environment And Occupational Health And Safety

Issues associated with the environment and OH&S are parallel to those of the requirements of physical containment classification and GMP. No significant additional obstacles are forecast.

Tissue Residues

Tissue residues are generally not considered significant with vaccines and no special considerations are present for the proposed vaccine.

Overall, there do not appear to be any significant hurdles to achieving registration once the manufacturing stages are successfully completed. However, the demands of compliance with GMP should not be underestimated and are more fully described in the next section.

Quarantine

For any imported biological materials, an import permit will be required from AQIS subject to their consideration of the risks associated with the import.

The only biological materials that may need to be imported include the vaccine master seed (Bacillus anthracis strain 34F2) and the virulent challenge strain (usually Bacillus anthracis JB17). We have confirmed that both of these strains are available and can be obtained from the Veterinary Laboratories Agency in the UK and neither is expected to be associated with any insurmountable quarantine risk.

The vaccine strain originated before the emergence of BSE and has been in use worldwide since 1978. Previous correspondence with AQIS has suggested that this import does not carry an unacceptable risk131.

The challenge strain, if imported, is for use in experimental animals in a secure PC3 facility where all materials will be incinerated. Under these conditions it is expected that this strain also will not carry an unacceptable quarantine risk.

The production of vaccine does not require any constituents of animal origin other than trypsin digests of casein, a product that is usually considered low risk.

Other Regulatory Requirements

A facility to produce anthrax vaccine at EMAI would also be subject to state planning laws, environmental controls and occupational health and safety requirements.

The Department of Primary Industries has indicated it would accept responsibility for construction including obtaining planning approval.

The use of anthrax vaccine is subject to approval by the Chief Veterinary Offer in each State. This would not change with vaccine produced at EMAI.

The local consent authority for the area is Wollondilly Council. Development consent would be required for the proposed facility.

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131 Correspondence from Robert Heard (Manager Animal and Plant Programs Branch, AQIS) to Richard Bevan (consultant) dated 10 August 2000. Robert Heard writes: " … I can confirm the following: that the anthrax master seed put down in 1967 by CVL and used by Websters in their anthrax vaccine from about 1992-1997 does not represent a quarantine risk with regard to: BSE, because it predates the first known cases by more than 8 years and Scrapie, because of the history of use in a vaccine produced and used in sheep within Australia for a number of years."

10.7 Manufacturing Considerations

10.7.1 Background Documents

In considering the local manufacture of an anthrax vaccine, reference has been made to the following principal documents:

ANTHRAX VACCINE MANUFACTURING DOCUMENTS

1. Anon (1962). Anthrax vaccine stock cultures. Production of spore concentrate. Methods in use January 1962.

2. APHIS, USDA (2004a). 113.45 Sheep safety test. 9CFR . Ch 1 (1-1-04), p 613

3. APHIS, USDA (2004b). 113.64 General requirements for live bacterial vaccines. 9CFR. Ch 1 (1-1-04), pp 620-621

4. APHIS, USDA (2004c). 113.66 Anthrax spore vaccine – non-encapsulated. 9CFR Ch. 1 (1-1-04), pp 622-623

5. APVMA (2005). Code of good manufacturing practices for veterinary chemical products. Draft for consultation.

6. British Pharmacopoeia (Veterinary) (2004a). Veterinary vaccines. Pp 154-160

7. British Pharmacopoeia (Veterinary) (2004b). Anthrax vaccine, living. Page 160.

8. EUDRA (2005a). The Rules Governing Medicinal Products in the European Union. Volume 7 - Guidelines : Veterinary Medicinal Products. General requirements for the production and control of live mammalian bacterial and viral vaccines for veterinary use (http://pharmacos.eudra.org/F2/eudralex/vol-7/B/7Blm1a.pdf)

9. EUDRA (2005b). The Rules Governing Medicinal Products in the European Union. Volume 7 - Guidelines : Veterinary Medicinal Products. Specific requirements for the production and control of bovine live and inactivated viral and bacterial vaccines (http://pharmacos.eudra.org/F2/eudralex/vol-7/B/7Blm4a.pdf)

10. EUDRA (2005c). The Rules Governing Medicinal Products in the European Union. Volume 7 - Guidelines : Veterinary Medicinal Products. Specific requirements for the production and control of ovine and caprine live and inactivated viral and bacterial vaccines (http://pharmacos.eudra.org/F2/eudralex/vol-7/B/7Blm6a.pdf)

11. European Pharmacopoeia (1997a). Anthrax spore vaccine (live) for veterinary use. 1997:0441, 399-400

12. European Pharmacopoeia (1997b). Vaccines for veterinary use. 1997:0062, 1698-1702

13. Misra RP (1991). Manual for the Production of Anthrax and Blackleg Vaccines, (FAO Animal Production & Health Paper No. 87) (Appendix 5)

14. OIE (2004). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.2.1. Anthrax.

15. Standards Australia (2002). Safety in laboratories. Part 3: Microbiological aspects and containment facilities. 5th Edition. AS/NZA 2243:3:2002 (Incorporating Amendment No. 1).

16. Sterne M. (1937). The effects of different carbon dioxide concentrations on the growth of virulent anthrax strains. Pathogenicity and immunity tests on guinea-pigs and sheep with anthrax variants derived from virulent strains. Onderstepoort J Vet Sci Anim Ind 9: 49-67

17. Sterne M. (1939). The use of anthrax vaccines prepared from avirulent (uncapsulated) variants of *Bacillus anthracis*. Onderstepoort J Vet Sci Anim Ind 13: 307-312

18. Sterne M. (1946). Avirulent anthrax vaccine. Onderstepoort J Vet Sci Anim Ind 21: 41-43

19. WHO (1966). General requirements for manufacturing establishments and control laboratories (Requirements for biological substances No.1) (WHO Technical Report Series, No. 323), pp .11-22

20. WHO (1967). Requirements for Anthrax Spore Vaccine (Live, for Veterinary use) (Requirements for biological substances No. 13) WHO Expert Committee on Biological Standardization. Nineteenth Report. (WHO Technical Report Series, No. 361, 1967), pp .31-40

21. WHO (1969a). Anthrax vaccine (live – for veterinary use). WHO Expert Committee on Biological Standardization. Twenty-first Report. (WHO Technical Report Series, No. 413, 1969), p 18-19

22. WHO (1969b). Seed culture of *Bacillus anthracis*. WHO Expert Committee on Biological Standardization. Twenty-first Report. (WHO Technical Report Series, No. 413, 1969), p 25

23. WHO (1979). Anthrax spore vaccine. WHO Expert Committee on Biological Standardization. Thirtieth Report. (WHO Technical Report Series, No. 638, 1979), p 16

10.7.2 Introduction To Current Standards

Conformance with the code of Good Manufacturing Practice is an obligation imposed by the APVMA on all manufacturers of animal health products.

The Australian code is similar to the European and United States codes. Indeed, the current Australian code is in the process of being replaced by one that closely matches that of the European Union, modelled on the Guide To Good Manufacturing Practice for Medicinal Products, Pharmaceutical Inspection Convention/Pharmaceutical Inspection Co-operation Scheme (PIC/S), 1 July 2004.

The implementation timetable seeks comments on the new draft code by 9 September 2005[132], introduction in January 2006 and auditing of compliance commencing in January 2007.

The new code is substantially more prescriptive than the current code and contains 28 quality objectives.

Discussions have been undertaken with the APVMA to assess the feasibility of various aspects relating to manufacturing.

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[132] Revisions to the APVMA's Manufacturing Principles and the Code of Good Manufacturing Practice for Veterinary Chemical Products. http://www.apvma.gov.au/qa/gmp_codes_drafts.shtml

For example, with respect to the issue of multiple uses of a live spore vaccine plant, this concept was considered possible but would require convincing demonstration of decontamination and absence of transfer to other vaccine products. If the other product were a diagnostic test for example, then the risks would be lower.

It was recommended that the design of the plant address the segregation of high and low risk production areas, to facilitate the approval of the use of low risk areas for other products.

When commissioning a new facility considerable support and assistance is available if requested and the following steps could be implemented:

1. Site plans: opinion can be sought from APVMA and/or APVMA GMP auditors.
2. Inspection of new facility by auditor (examine site, facility, documentation) and advise APVMA whether facility likely to satisfy GMP requirements.
3. APVMA issues conditional licence, which may permit a number of validation batches to be produced (but not sold).
4. Facility re-inspected by auditor and batch records and validation data examined.
5. APVMA issues new licence permitting sale.
6. Ongoing audits according to standard practice.

It was emphasised that many tasks could be pre-validated prior to vaccine production and that this may save time (for example, media filling, aseptic processing etc).

It was highlighted that the requirements of GMP are demanding and in the experience of the APVMA, government departments involved in manufacture often struggle to comply with the documentation and other requisites of GMP. The APVMA is aware of a number of cases of manufacturing being transferred to other organisations or facilities closing due to failure to meet the demands of GMP[133]. While it is not impossible to comply with GMP, care needs to be taken in the selection and training of personnel and in the close supervision and documentation of the manufacturing process.

Education, training and culture are considered of great importance in successful compliance. It was considered that as a minimum, the leader of the manufacturing team should be very experienced in GMP, manufacturing and QA.

Given due consideration of the prevailing standards (see next section) and commitment to quality management, no insurmountable hurdles were foreseen by the APVMA in having a new plant commissioned and licensed.

10.7.3 Quality Standard Of The Manufacturing Operation

While the technology of production of the Sterne anthrax vaccine has not changed since first described in 1937, the environment in which the vaccine is manufactured bears little resemblance to that which existed during those early years. Quality assurance and the production of a reliable and reproducible product are paramount to successful production.

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[133] For example, Gazette APVMA 10, 4 October 2005 page 48:

LICENCE SUSPENSIONS: (The APVMA has suspended the following licences under section 127(1) of the Agricultural and Veterinary Chemicals Code ['the Agvet Code]). Department Of Primary Industries, Water And Environment, Tasmania

Stringent regulatory requirements for documentation and validation of every step in the manufacturing process mean that consumers can have increased confidence in the efficacy, safety and quality of those products they elect to use. However, the new quality standards introduce significant complexity and increases in costs of production.

In August 2005 the Australian Pesticides and Veterinary Medicines Authority introduced for public consultation a draft Code of Good Manufacturing Practice for Veterinary Chemical Products. It is anticipated this will be finalised and implemented by 1 January 2006.

The revised draft cGMP places greater emphasis on the need for manufacturers to maintain an effective QA function and to periodically undertake a scientifically based risk assessment of their processes and procedures and to adopt a culture of continuous improvement.

Furthermore, responsibility for Quality and Production, the code states (page 14, paragraph 106), must be allocated to specific persons who are suitably qualified, trained or experienced. Normally, these key positions should be occupied by full-time personnel (page 17, paragraph 207). The person responsible for Quality must have the necessary independence and authority to ensure that quality measures are employed in the production and testing of product and that product is not released until it has been judged to be satisfactory.

The INTRODUCTION to the APVMA cGMP encapsulates the objectives of quality production as follows:

> *Veterinary medicines are subject to a registration process that requires them to be fit for their intended use and not place treated animals or users at risk due to inadequate safety, quality or efficacy. Veterinary chemical products must be manufactured in such a way to ensure that they comply with their registered particulars and that there is batch-to-batch consistency.*
> *The ultimate responsibility for attaining these quality objectives lies with senior management, but their achievement also requires the participation and commitment of all staff, at all levels, within the organisation. In order to achieve these objectives, the manufacturer must have in place a comprehensively designed, adequately resourced and correctly implemented system of quality assurance incorporating the principles of Good Manufacturing Practice (GMP).*
>
> ***Quality Assurance (QA)** is a wide-ranging concept covering all aspects of the manufacturing process that individually or collectively influence the quality of a manufactured product. It is the sum total of the arrangements made to ensure that veterinary chemical products are consistently manufactured in an appropriate manner to the quality standards required for their intended use. Quality Assurance therefore incorporates both Good Manufacturing Practice (GMP) and Quality Control as well as other factors outside the scope of this GMP Code such as environmental and occupational safety controls.*
>
> *Quality Assurance requires manufacturers to have in place a quality management system encompassing the organisational structure, responsibilities, procedures, instructions, processes and resources necessary for implementing quality management. That quality system must ensure that facilities and equipment are suitable for the type of products made, that there are sufficient competent personnel and that appropriate procedures are in place to ensure appropriate quality standards are met. In addition, the system must ensure that all materials involved in the manufacturing process (including raw materials, intermediate materials or samples from any material relevant to product quality) are checked and tested where necessary, to ensure that they meet required quality standards before they are*

released for use. Procedures must be in place to ensure that finished product has been made correctly and meets all required quality tests before it is released for supply or sale.

The quality system must be relevant to the needs of the product. It must be fully documented, monitored for effectiveness and incorporate an element of continuous improvement.

***Good Manufacturing Practice (GMP)** is that part of Quality Assurance which ensures that products are consistently manufactured to the quality standards appropriate to their intended veterinary use and in accordance with their registration particulars and specifications. Good Manufacturing Practice is concerned with both production and quality control. It is a means of giving consumers confidence that the products meet required quality standards and are safe and reliable for the purposes for which they are intended. The basic requirements of GMP are that:*

(a) all manufacturing processes are clearly defined, systematically reviewed in the light of experience and shown to be capable of consistently manufacturing veterinary chemical products of the required quality and complying with their specifications;

(b) critical steps of manufacturing processes and significant changes to the processes are validated;

(c) all necessary facilities for GMP are provided including:

(i) appropriately qualified, trained or experienced personnel;

(ii) adequate premises and space;

(iii) suitable equipment and services;

(iv) correct materials, containers and labels;

(v) approved procedures and instructions;

(vi) suitable storage and transport;

(d) instructions and procedures are written in an instructional form in clear and unambiguous language, specifically applicable to the facilities provided;

(e) operators are trained to carry out procedures correctly;

(f) records are made manually and/or by recording instruments during manufacture which demonstrate that all the steps required by the defined procedures and instructions were in fact taken and that the quantity and quality of the product was as expected. Any significant deviations are fully recorded and investigated;

(g) records of manufacture, including distribution, that enable the complete history of a batch to be traced, are retained in a comprehensible and accessible form;

(h) a system is available to recall any batch of product from sale or supply; and

(i) complaints about marketed products are examined, the causes of quality defects investigated and appropriate corrective and preventative measures are taken in respect of the defective products and to prevent re-occurrence.

***Quality Control** is that part of GMP which is concerned with sampling, specifications and testing, and with the organisation, documentation and release procedures which ensure that the necessary and relevant tests are carried out and that materials are*

not released for use, nor products released for sale or supply, until their quality has been judged to be satisfactory.
Compliance with GMP ensures that quality is built into the product at the time of manufacture. It requires products to be consistently manufactured in a safe and clean environment, by specified methods, under adequate supervision, with effective quality control procedures, and with a documentation trail that links starting materials, through the various manufacturing processes, to the finished product.

As described in detail in the draft cGMP, the key components of GMP include the following:

QUALITY MANAGEMENT

Manufacturing Principles; Quality Management Guidelines; Management of Quality; Quality Assurance; Quality Control; Quality Assurance and Production Nominees; Process Control and Change Control

PERSONNEL AND TRAINING

Manufacturing Principles; Personnel and Training Guidelines; Key Personnel; Qualifications and Experience; Training and Competency Assessment; Personal Hygiene/Health Issues

BUILDINGS AND GROUNDS

Manufacturing Principles; Buildings and Grounds Guidelines; Cleaning and Sanitation; Storage Areas (including Receipt and Dispatch); Production Areas; Quality Control Areas; Ancillary Areas; Animal Houses

EQUIPMENT

Manufacturing Principles; Equipment Guidelines; Equipment Qualification and Validation; Maintenance; Calibration; Cleaning

DOCUMENTATION

Manufacturing Principles; Documentation Guidelines; Document Control; Records; Documents Required; Specifications; Materials Control (Stores Receipt, Storage and Disposal); Master Manufacturing Formula, Batch Records and Processing Instructions; Master Packaging and Labelling Instructions; Batch Manufacturing/Processing Records; Batch Packaging Records; Quality Control Sampling and Testing; Release/Rejection and Distribution of Finished Product; Complaints, Recalls and Returns; Computer Records; Laboratory Records; Validation records

COMPUTER SYSTEMS

Manufacturing Principles; Computer Systems Guidelines

PRODUCTION

Manufacturing Principles; Production Guidelines; Materials Control (including Storage); General; Receipt, Storage and Quality Assurance of Raw Materials; Receipt, Storage and Quality Control of Packaging Materials; Cross-Contamination Control; Process Validation; Production Procedure; Dispensing of Raw Materials; Processing Operations; Intermediate and Bulk Products; Process Water; Primary (Filling) and Secondary Packaging Operations; Release of Finished Products; Residual, Rejected, Recovered and Returned Materials

QUALITY CONTROL

Manufacturing Principles; Quality Control Guidelines; Documentation; Sampling; Sampling Plans; Sampling Procedures; Retention Samples; Product Release

CONTRACT MANUFACTURE

Manufacturing Principles; Contract Manufacture Guidelines; The GMP Agreement; The Contract Giver; The Contract Acceptor; Inspection of Contract Manufacturers

INTERNAL AUDITS

Manufacturing Principles; Self Inspection Guidelines

COMPLAINTS AND PRODUCT RECALLS

Manufacturing Principles; Complaint and Product Recall Guidelines

MANUFACTURE OF STERILE PRODUCTS

Manufacturing Principles; Introduction; Premises; Production Areas; Sanitation and Hygiene; Environment Control; Air Quality; Environmental Monitoring; Personnel; Equipment; Specification for Materials; Processing; Sterilisation by Heat – General; Sterilisation by Moist Heat; Sterilisation by Dry Heat; Sterilisation by Radiation; Sterilisation by Ethylene Oxide; Sterilisation by Filtration; Finishing (Primary Packaging); Quality Control; Aseptic Processing; Premises; Production Areas; Sanitation and Hygiene; Environment Control; Personnel; Equipment; Processing

IMMUNOBIOLOGICAL PRODUCTS

Manufacturing Principles; Essential Information; Premises; Segregation and Containment; Sanitation, Disinfection and Waste Disposal; Personnel; Equipment; Animals and Animal Houses; Production; General ; Starting Materials; Media; Seed Lot System; Operating Techniques; Quality Control

10.7.4 Master Seed Organism

A master seed (*Bacillus anthracis* $34F_2$) is required from which the vaccine can be produced. There are three potential sources for this.

1. The worldwide parent culture for Sterne $34F_2$ strain of *B anthracis* is held by the Veterinary Laboratory Agency (VLA) in Weybridge. It was established long before the occurrence of BSE and, subject to satisfying AQIS data requirements, is understood to be potentially importable into Australia.

   Full history is generally required although previous correspondence with AQIS[134] suggests that there will be no major impediments to import of the master seed. The current charge by AQIS for a Category 5 import (which applies to a single new master seed for *in vivo* use) is $340[135].

2. CSL (now Pfizer) imported master seed from this source in 1988, using it to manufacture vaccine before production was discontinued. It is understood Webster used the same master seed source for the production of its vaccine.

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[134] Correspondence from Robert Heard, op cit.

[135] Charging Schedule for Import Permit Applications. AQIS Import Permit Application Fees. Webpage last reviewed 27 July 2005.

The CSL master seed is still available in Australia, although its viability and potency are unknown. Pfizer has indicated it would make this available to an industry consortium responsible for re-establishing vaccine production, without limitation or royalty.[136]

There may be some time saving in resurrecting the CSL master seed if it can be shown to be the one that was in use when CSL manufactured and sold anthrax vaccine.

3. The anthrax vaccine currently marketed by Fort Dodge Animal Health also offers a potential source of a master seed.

   Culturing of spores from a sample of vaccine followed by verification of compliance with the pharmacopoeial standards for purity, identity, potency and safety could yield a valid parent culture for division into a seed programme.

10.7.5 Production Personnel

10.7.5.1 Personnel Requirements

To ensure compliance with the prevailing cGMP the personnel required to support the manufacturing operation will need to match the following profiles.

POSITION	PROFILE
Manufacturing Manager	o Overall responsibility for recruitment and training of staff, and the planning and implementation of each manufacturing campaign, including compliance with regulatory requirements (GMP, OHS, EPA, etc) o Needs to have recognised experience and expertise in bacterial vaccine manufacture in Australia. o Must understand specialised technical, quality and legal requirements relating to the manufacture of veterinary chemical products. o Responsible for regular and systematic internal audits o Ensure that products are produced and stored according to documented procedures; o Approve procedures relating to production operations and ensure their strict implementation; o Approve and monitor any sub-contracted production work; o Ensure that production records are evaluated and signed by an authorised person before they are submitted for product release; o Ensure that production areas, premises and equipment are maintained to an appropriate standard; o Ensure that appropriate validations are conducted; and o Ensure that initial and continuing training of production personnel are conducted, according to need.

[136] Pfizer Animal Health (Australia), personal communication.

Quality (Assurance / Quality Control) Manager

OVERALL RESPONSIBILITY

- Responsible for monitoring and documentation of all manufacturing procedures (ie the continuous process from receipt of materials to testing, labelling, storage and despatch of finished products) and ensuring manufacturing processes comply with all regulations, especially GMP.

PROFILE

- Experience in vaccine manufacturing QA.
- Science background with qualification in microbiology.
- Meticulous and systematic documentation required.
- Senior role with high level of responsibility

KEY FUNCTIONS

- Evaluate and authorise master manufacturing and packaging documents;
- Approve specifications, sampling instructions, test methods and other QC procedures;
- Approve or reject raw materials, packaging materials, and intermediate, bulk and finished products;
- Review completed batch records as part of the release procedures;
- Ensure that all necessary testing is carried out;
- Approve and monitor any contract analysts;
- Monitor quality performance of any sub-contract manufacturers;
- Check the maintenance of the quality control area, premises and equipment;
- Ensure that appropriate validations are conducted;
- Ensure that initial and continuing training of quality control personnel are conducted, according to need;
- Approve and monitor the suppliers of materials.
- Establish adequate quality control specifications for all materials at all stages of manufacture and for the finished product;
- Establish, document, validate and implement all quality control test procedures;
- Assess and release/reject starting and intermediate materials for each batch;
- Assess and release/reject each batch of finished product for supply;
- Keep reference/retention samples of materials and products;
- Ensure the correct labelling of containers of materials and products;

	o Monitor the suitability of packaging materials; o Monitor the stability of the products, the suitability of expiry dates and product storage conditions; o Participate in the investigation of complaints related to the quality of the product; o Monitor environmental control of QC laboratories and test animal houses as appropriate; o Review trends in analytical results or yields; o Establish or approve procedures for animal quarantine, animal testing and the recovery of biological material from animals for use in analysis, testing or production.
Technical Officers (up to 3 at various stages of production) (Report to Manufacturing Manager)	o Responsible for implementation of manufacturing plan prepared by Manufacturing Manager. o Create, update and follow Production Standard Operation Procedures (SOPs) o Record keeping o Decontamination (before, during and after each campaign) o Air quality monitoring o Equipment testing and validation o Microbiology and environmental monitoring o Ordering materials o Receipt of materials o Preparing materials for manufacturing o Maintenance and testing of seed-lots o Production processes (preparation of vaccine concentrate [inoculation, harvesting, purity testing, glycerination]) o Product testing (bacterial contamination, viable spore numbers, safety testing, potency testing) o Dilution of vaccine concentrate o Filling and packaging o Control tests on final product o Labelling o Storage
Regulatory Affairs	o Part of the role of the Manufacturing Manager or contracted to external consultant. o Principal objective is keeping abreast of changes in regulatory requirements that have any impact on the manufacturing operations (eg GMP, Security, Environmental, OHS, Dangerous Goods, etc)

There are three general scenarios under which personnel for producing anthrax vaccine at EMAI could be obtained. In order of increasing feasibility these include use of EMAI staff, use of outside casual staff and third party production under contract.

10.7.5.2 Use of EMAI staff

This is the option preferred by the IWP and is based on the assumption that EMAI staff, being trained in laboratory and microbiological techniques, could readily learn the procedures required for producing vaccine. Moreover, being located at the site, it is anticipated they would work on vaccine-related tasks for only as long as required, returning to their usual activities when finished.

It seems reasonable to expect this would result in a lower cost of production than having external personnel available fulltime or permanent part time, particularly if there was no minimum or standing cost involved.

However, there is significant doubt as to whether suitable staff could be freed from other activities when required to make vaccine. As discussed earlier, nearly all personnel at EMAI are project-funded and not readily available for reassignment. The problem would particularly limit the potential for several staff to be available at once and may be of acute significance if there was an urgent need to produce vaccine to deal with an outbreak.

Even if that obstacle were overcome, EMAI staff would require substantial training to be able to comply with the code of Good Manufacturing Practice plus regular refresher training to maintain compliance. This essentially requires the same people to be involved each time - it would certainly not be feasible for different staff to be used each time vaccine was produced.

Inevitably, EMAI staff could only operate under experienced supervision.

10.7.5.3 Outside casual staff

A variation on the first scenario envisages the employment of an experienced manufacturing manager who would be responsible for obtaining suitable personnel to produce vaccine on the most economical basis possible, from wherever they could be found.

These might include suitable EMAI staff if available. However, they could also be former manufacturing staff from another vaccine or pharmaceutical manufacturer who are familiar with microbiological techniques and GMP and available to work on a casual basis. It is considered reasonably likely that such people would be available within commuting distance of EMAI.

The intention would be to establish a permanent casual workforce that was familiar with production of the vaccine and available to work as required. This would help to maintain GMP while also minimising production costs and batch failures.

10.7.5.4 Production by a third party, under contract

It may be possible to secure the services of a commercial vaccine manufacturer to produce anthrax vaccine at the EMAI facility, using the manufacturer’s trained and experienced staff brought in for the purpose.

Although such personnel are likely to require some help with the specifics of anthrax vaccine production, they would be familiar with the principles of GMP and the production of other types of vaccines. Moreover, for commercial reasons, inexperienced staff are not likely to be used.

Fort Dodge has indicated that it would be interested in this opportunity on a fee for service basis. The basis of calculating the fee would be the total cost of labour including related office overheads (eg payroll), plus around forty percent margin.

Intervet also expressed interest in principle, viewing it as both an opportunity to give staff wider experience and a socially useful activity. However, being located in Victoria, there may be practical constraints regarding travel and accommodation.

The other two veterinary vaccine companies with facilities in Sydney, Bioproperties and Allied Animal Health, expressed some reluctance. Both are much smaller and the impact on their existing activities would be much greater.

Pfizer was not approached but its position is considered likely to be similar to Intervet.

10.8 Intellectual Property

Under the EMAI scenario, intellectual property may be created in the form of the regulatory data package (copyright plus data protection) and in the label design (copyright and trademark).

There would be no obstacle to the consortium retaining ownership of any such intellectual property, if it wished.

Even if it were decided to transfer vaccine production to another entity, such as a commercial vaccine producer, ownership of intellectual property could be retained by making it available under licence.

10.9 Entity and Management

10.9.1 Entity Options

The entity through which anthrax vaccine could be manufactured and sold is anticipated to require the following characteristics:

1. The ability to acquire, hold and dispose of property including land
2. The ability to borrow money
3. The ability to enter into contracts including lease or tenancy agreements
4. Limited liability for members/shareholders, directors and officers
5. The ability to sue and be sued
6. Perpetual succession
7. The ability to trade in any State
8. The ability to reflect unequal equity interests.

A standard proprietary company limited by shares would achieve all of these outcomes while also offering significant flexibility for the future. It is probably the most obvious and logical option.

Nonetheless, assuming the vaccine would not be sold with the primary intention of generating a profit to be distributed to shareholders, there are other options. The full range of options is as follows:

- Incorporated association
- Registered cooperative
- Company limited by guarantee
- Company limited by shares (private or public)
- Corporation established under statute

10.9.1.1 Incorporated Association

Description: Established under State legislation (in NSW, the *Associations Incorporation Act 1984*).

Cannot be formed for the primary purpose of earning a profit. If it does earn a profit, it may not be distributed amongst the members.

Committee members owe analogous common law fiduciary duties to the association and its members as are imposed on company directors.

Requires a minimum of five members (with no upper limit). The model rules provide for a minimum of seven committee members. Members have a vote of equal value.

Reporting and compliance obligations are somewhat less than for a company.

Incorporated associations are not recognised nationally. To carry on business in other states they must either incorporate under equivalent legislation (this can cause some confusion as the legislation is not uniform) or register with ASIC under the Corporations Act and be allocated an "Australian registered body number" ("ARBN").

Due to the limited duties of audit and disclosure, groups that would otherwise be eligible for registration as an incorporated association may be queried or refused if they are perceived to involve large amounts of money.

Assessment: As Association involves minimal administration but the interstate limitation may make it somewhat inflexible. Moreover, member safeguards are not high.

10.9.1.2 Cooperative

Description: Cooperatives are established under State legislation (in NSW the *Cooperatives Act 1992*).

Cooperatives can be registered as trading cooperatives and may distribute profits to members. Reporting and compliance is comparable to an incorporated association although trading cooperatives must have their accounts audited.

There must be at least five members (with no upper limit). Each member of the cooperative has a vote of equal value.

The number of directors is not fixed although the Registry recommends at least three. Obligations are comparable to those imposed on company directors.

The Act requires that cooperative rules specify the manner and extent to which a member is required to actively support it. This support is measured against the cooperative's 'primary activity' (or a combination of primary activities). Members are obliged to use or support the activities of a cooperative in order to retain the right to be a member, including the right to vote.

Cooperatives are not recognised nationally. They must obtain “foreign” registration as a cooperative in other states to carry on business there.

Assessment: The obligatory auditing obligation is more onerous than for a private company. Membership may also not suit some consortium members.

10.9.1.3 Company Limited By Guarantee

Description: A company limited by guarantee tends to be used by non-profit organisations that deal with large sums of money or conduct substantial trading activities.

It may operate throughout Australia but cannot distribute its profits to members. Directors are subject to the duties and obligations in the Corporations Act.

There are no shareholders or share capital. The minimum number of members is one. Each member has a vote of equal value.

A company limited by guarantee is a public company and therefore subject to greater regulation and reporting than a private company. Establishment costs are higher, the company must have three directors and a secretary and accounts must be audited and lodged with ASIC. It also requires a registered office which must be open to the public at least three hours between 9am and 5pm.

Assessment: As a public company, a company limited by guarantee carries more onerous compliance obligations than a private company with no significant benefits.

10.9.1.4 Company Limited By Shares

Description:

Private

A private (proprietary) company is designed for a limited group of shareholders who do not wish to invite the public to subscribe share capital or lend money to the company and wish to restrict transfer of its shares. The vast majority of business in Australia is conducted via private companies.

Private companies may have between one and 50 shareholders. The minimum number of directors is one. They cannot offer shares or security to the public but may offer shares to existing shareholders or employees. They may operate throughout Australia.

Each member (shareholder) has a vote equal to the number of shares held. Profits may be distributed to members in accordance with their shareholding.

Directors are subject to the Corporations Act. Accounts do not need to be audited.

Sale or transfer of shares can be restricted by the Constitution to existing shareholders.

Public

Public companies require at least three directors and a secretary, have no upper limit on shareholder numbers and may offer shares to the public.

Compliance and reporting costs are higher than for private companies. Accounts must be audited and lodged with ASIC and there must be a registered office open to the public for at least three hours a day.

Assessment: A public company has high compliance and reporting obligations with no advantage relative to a private company in this situation. A private company allows substantial flexibility while safeguarding shareholder interests.

10.9.1.5 Statutory Corporation

Description: The *McGarvie Smith Institute Incorporation Act 1928 (NSW)* converted the McGarvie Smith Institute into an incorporated body. This effectively prevented any takeover by a competitor seeking to obtain access to the Institute's technology and also formalised the government's obligation to pay interest on funds contributed by McGarvie Smith.

More recent examples of statutory corporations in NSW include the various aboriginal land councils, the Children's Commission, the WorkCover Authority, Sydney Water, the Macedonian Orthodox Church Property Trust and the Greyhound Racing Authority.

A statutory corporation could be established under state (but not commonwealth) legislation in this instance as well, if it was considered that other options were not adequate. The corporation could be made subject to or exempt from any elements of the Corporations Act, or any other relevant legislation. A corporation not registered under the Corporations Act may be restricted from trading in other states unless registered with ASIC.

The principal benefit of a statutory corporation is that its role is defined by legislation and cannot be altered. Governance and limits on activities are also generally defined.

The main disadvantage is the potential for inflexibility, with legislative amendment required if change becomes necessary. A statutory corporation established to produce anthrax vaccine might be unable to enter into commercial arrangements such as joint ventures or outsourcing, for example, unless such options were envisaged when the legislation was drafted.

Assessment: The advantages of a statutory corporation are unlikely to be relevant to this project, while the inflexibility may prove to be a significant hindrance.

10.10 Governance and Management

The governance and management of an entity that manufactured and sold anthrax vaccine at EMAI would require the following:

1. Clear separation of operational aspects from policy and political concerns.
2. The ability to reflect diverse stakeholder interests.
3. A means of resolving differences between stakeholders with respect to policy issues.

The normal corporate approach to achieve these outcomes is to appoint a Chief Executive who reports to a board. Members of the board may, but need not, represent particular shareholder/stakeholder interests.

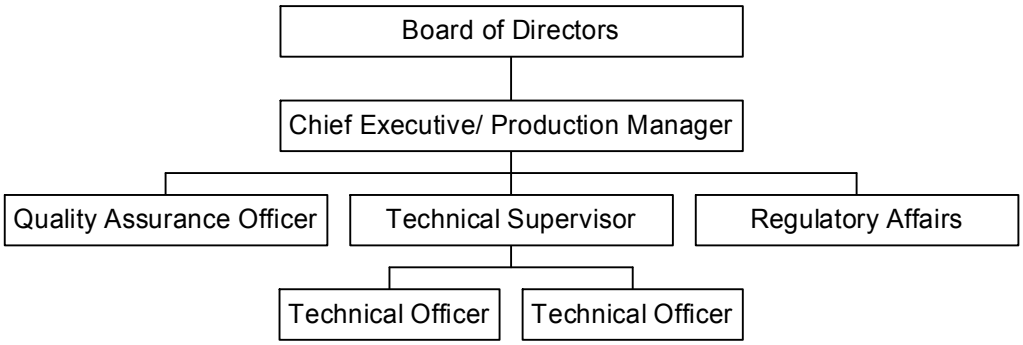
In this project a board of between three and six directors would seem appropriate, nominated by consortium members with additional stakeholders such as NSW Department of Primary Industries. If a major motivation for local anthrax vaccine manufacture was to prevent the consequences of agri-terrorism, a representative nominated by the National Security Committee may also be warranted.

Given the economics of the project, it is expected that directors would receive no remuneration apart from out of pocket expenses.

Management of production and marketing of the vaccine would be under the control of the Chief Executive, subject to objectives, policies, budgets and strategies agreed by the board. The Chief Executive would have responsibility for all steps necessary to implement the strategies and achieve the objectives.

To ensure that stakeholders retain an appropriate level of involvement, it may be preferable for the Chief Executive to participate on the board *ex officio* rather than as a director.

A possible organisational structure is shown below. It is unlikely, given both the duties and economics of the project, that any positions would be full-time. That includes the Chief Executive. The Regulatory Affairs function could be provided by the Chief Executive/Production Manager if the incumbent was appropriately qualified and experienced. Alternatively, it could be outsourced to a consultant.



10.11 Risk Management

10.11.1 Risk Identification

Risks associated with the EMAI proposal are listed as follows. Peter Claxton identified the majority of these in his report.

Risk	Insurable	Comment
Inability to supply	No	Risk applies to livestock industry, not consortium
Product contamination	Yes	Product liability cover
Lack of efficacy	Yes	Product liability cover
Lack of safety	Yes	Product liability cover
Inadequate storage/ mishandling	Yes	Product liability cover (but no liability once product changes ownership)
Improper administration of vaccine	No	Risk to livestock owner (through lack of efficacy) but not consortium
OH&S	Yes	Workers compensation cover
Accidental self injection by users	No	Risk to user only. Label warning may be warranted.
Negligence by directors or officers	Yes	Directors and Officers cover
Insolvent trading	No	Risk to directors, cannot be avoided
Employee theft	Yes	Fidelity guarantee (usually bundled with Directors and Officers cover)
Unauthorised pollution of environment	Yes	May be bundled with product liability

10.11.2 Risk Mitigation

Aside from implementing international best practice in relation to manufacture of the vaccine and related operations, the principal means of mitigating risk is via insurance.

The main concern is product liability. At least in theory, there are four ways in which this risk could be mitigated:

10.11.2.1 Self-insurance

There is no obligation, regulatory or otherwise, to carry product liability insurance. Self-insurance amounts to the consortium opting to fund successful claims out of its own resources.

The risk is that, in the event of a successful action, the assets of the consortium may be vulnerable to liquidation to settle the debt. Moreover, directors may become personally liable if the organisation continues to trade following a judgement against it that cannot be paid.

Self insurance is unmistakeably the lowest cost option provided there are no claims. Claxton reported that there did not appear to have ever been a claim in Australia seeking damages arising from the manufacture or distribution of anthrax vaccine[137].

Nonetheless, it is not generally regarded as prudent to self-insure unless the organisation has substantial assets that would enable it to withstand a major claim.

To overcome that, one or more of the shareholders could provide a guarantee up to the limit of agreed cover. The limit would typically be the same as a formal insurance policy (eg $10 or $20 million).

Shareholders are generally reluctant to provide such guarantees and it is considered unlikely to be feasible in this case.

10.11.2.2 Indemnity by a third party

This involves an independent third party agreeing to cover any successful claims against the consortium.

The main alternatives in this project are considered to be the NSW government, Commonwealth government and Meat and Livestock Australia.

We understand all three have ruled out any such indemnity. Moreover, Meat and Livestock Australia has confirmed that its existing insurance does not include product liability. Unless there was a change of attitude by at least one of the three, this does not appear to be a practical option.

10.11.2.3 Manufacture by a third party

If a third party were to undertake manufacture of the anthrax vaccine at EMAI, there is a good chance the vaccine could be brought under the manufacturer's existing product liability cover. This would most likely require it to be sold under the manufacturer's label (although not necessarily as registrant or owner of other intellectual property).

Provided the insurer had no concerns about the use of the EMAI facility, there is not likely to be any increase in the premium to pass on to the consortium.

However, it would require the third party to be fully responsible for production of the vaccine so that it was not exposed to risk that it did not manage. This would most likely cover everything from sourcing raw materials through to finished goods storage.

Aside from the cost implications of using a third party, this option is practical.

10.11.2.4 Product liability insurance

Claxton comprehensively investigated the availability and cost of product liability insurance in 2004[138]. He found that cover of $10 million would cost between $87,500 and $109,000. Cover of $20 million was about $15,000 extra.

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[137] Claxton 2004 Op cit

[138] Claxton 2004 Op cit

Our investigations essentially confirmed Dr Claxton's findings. Cover would certainly be obtainable at a price.

Quotes obtained via two brokers (one with assistance of the IWP) indicate cover of $20 million would cost $65,000 to $150,000 per annum through underwriters located in the United Kingdom. Cover at higher cost (more than $200,000) would be available from other underwriters.

The brokers suggested the insurance market may have softened since 2004 and that extra information and a claim-free period may lead to some reduction in the premium. However, estimates of the potential reduction varied. One suggested the cost may only fall by about 10% while another suggested it was unlikely to ever fall below $40-50,000.

The excess payable on each claim would be in the range of $10,000 - $50,000.

10.12 Distribution

A vaccine produced at EMAI would require a system for making it available to users, where and when required. Some variations from the current system are inevitable.

10.12.1 Current Situation

The use of anthrax vaccine is subject to Chief Veterinary Officer approval in all states. In practice this is relevant only in Victoria, NSW and Queensland, where the vaccine is used.

The distribution, sale and use of the vaccine is the same in NSW and Queensland, but significantly different in Victoria.

In NSW and Queensland, the vaccine is purchased by livestock owners via the rural merchandise network (eg Elders, Landmark, CRT) or from veterinarians who purchase through veterinary wholesalers.

Properties on which an anthrax case occurs are quarantined and must vaccinate susceptible livestock annually for a period of three years[139]. Quarantine continues for six weeks after vaccination.

Fort Dodge accepts orders from rural retailers (eg Elders, Landmark, etc) and veterinary wholesalers (eg Provet, Lyppards, etc) if accompanied by a completed “Approval to Obtain and Used Imported Anthrax Vaccine” form. These require the signature of the Chief Veterinary Officer or his delegate (generally the District Veterinarian).

Fort Dodge invoices the rural retailer or veterinary wholesaler, which in turn charges the livestock owner or veterinarian.

The approval form requires the livestock owner to observe the meat-withholding period of 42 days and report any adverse reactions to the District Veterinarian. Feedlots that vaccinate routinely must report vaccine use on a monthly basis to the District Veterinarian.

The livestock owner may administer the vaccine.

The NSW Department of Primary Industries previously held a reserve of vaccine at EMAI. However, as of early 2005 this had been fully utilised and was not replaced as Fort Dodge provided assurances that it always carries at least 20,000 doses.

A reserve of 800 doses of vaccine is also held at Dubbo RLPB to meet urgent requests and deal with minor outbreaks, mainly on weekends. This is replaced by new vaccine ordered and paid for by recipients.

In Victoria there is a regulatory requirement for anthrax vaccine to be administered by a veterinarian[140]. Apart from that, vaccine to be used in livestock for live export is distributed and sold in the same way as in NSW and Queensland. Fort Dodge will supply orders from veterinary wholesalers or rural retailers when accompanied by a completed “Application and Authority to Purchase and Administer Anthrax Vaccine in Victoria for livestock export”.

The situation is different for livestock not intended for export. Following outbreaks, the Victorian Department of Primary Industries enters into an agreement with the livestock owner to pay for vaccination, including veterinary costs to administer the vaccine. The owner is not released from quarantine until an agreement is executed. Most agreements are for three years although a shorter period can apply.

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[139] Agfact AO.9.24 (third edition) August 2004 NSW Department of Primary Industries.

[140] Livestock Disease Control Regulation 1995, Clause 26 (Victoria)

If the livestock owner wishes to continue to vaccinate after the agreement has expired, the Department will supply the vaccine but the owner must pay for a veterinarian to administer the anthrax vaccine. Few owners continue to vaccinate, apparently because of the impact on milk yield (there is a label warning about this).

Discussions with veterinarians within Victorian Department of Primary Industries (DPI Victoria) revealed that even in the shadow of the Tatura incident in 1997, neighbouring dairies were very reluctant to vaccinate unless compelled.

DPI Victoria maintains a stock of vaccine of approximately 20,000 doses to supply livestock owners.

The following details relevant to sale and distribution of the vaccine were obtained from Fort Dodge. They relate to the period June 2003 to December 2004.

1. For the 2003-2004 financial year there was a total of 96 orders. By contrast, for the last six months of 2004 there were 105 orders.

2. In total, these orders were sent to 68 separate locations. This represents the number of customers for the period.

3. The number of orders per month averaged 11, with a range of four to 23.

4. The median order size was 10 x 50 dose packs with an average of 52 and a range of one to 800.

5. 86% of orders were for NSW, 11% were for Queensland and 3% for Victoria.

10.12.2 Industry Working Party Preference

The Industry Working Party (via Peter Carter) has previously expressed a preference for distributing locally produced vaccine via the Rural Lands Protection Boards. This has no impact on the security of vaccine supply or the feasibility of local production. However, it was suggested it might contribute to a reduction in the price of the vaccine and perhaps other attractions to livestock producers.

The broad terms of this arrangement were set out in correspondence between Peter Carter and the State Council of Rural Lands Protection Boards[141]. The scheme envisaged storage of finished vaccine at the RLPB cold store at Dubbo from where it would be consigned to customers.

During the course of the project Peter Carter was advised by the RLPB State Council that this would only be possible on a full cost recovery basis. Those costs, as estimated, would make the proposal uneconomic.

It was therefore suggested that vaccine produced at EMAI simply required a distribution approach that suited livestock producers and was no more expensive than the method of distribution used for the current vaccine.

10.12.3 Potential Solution

Following is a suggested option for distribution of vaccine produced at EMAI which is thought likely to be acceptable to the IWP. It assumes there is no commercial entity involved in production that could manage distribution.

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[141] Letter dated 7 June 2005 from Peter Carter to Steve Orr, Chief Executive of the RLPB State Council.

1. Finished vaccine would be held at EMAI.

2. Orders would be placed via a consortium member such as ALFA or NSW Farmers.

3. To avoid the need for credit control, orders would be shipped once payment was received.

4. Orders would be packed and dispatched from EMAI when notified of confirmation of payment.

5. The vaccine would be shipped in a cool box such as a polystyrene container, possibly with dry ice. These items would be held in stock or readily accessible.

6. The frequency with which orders were shipped from EMAI would be a policy and cost decision by the consortium. Batch processing of orders (once a month, for example) may be possible if RLPBs carry stock.

7. RLPBs could, if they wish, order vaccine on the same terms as other distributors of vaccine.

8. Farmers and feedlot operators would order vaccine either via their normal distribution outlet or an RLPB. Interstate customers would continue to order as at present.

9. Advice of the new source of vaccine and its price would be notified to distributors, RLPBs and interstate customers.

10. A procedure for investigating lost orders and replacing goods damaged in transit would be needed. This would be a matter for the consortium member responsible for taking orders.

Under this scenario, the EMAI consortium would retain ownership of the vaccine until it was sold to a distributor or RLPB.

The principal risk is that stock may get lost in transit to customers. This can be mitigated by insurance.

Other distribution options are also available.

One would be to utilise a commercial company, either on a percentage or fee for service basis, to distribute the vaccine to rural merchandise retailers. It is conceivable that one of the animal health companies would be interested in using it as a lever to gain access to the major distributors, with a commercial return of minor importance.

In that case, depending on the economics, the distributor may be willing to offer credit to customers. Certainly the distributor would utilise its existing ordering system including the procedures for tracing lost orders.

Based on similar schemes elsewhere, it is anticipated that the cost of handling the vaccine in this manner would be approximately fifteen percent of sales.

Due to the range of possibilities in this area, no allowance has been made in the cost estimates for distribution.

10.13 Complaint Handling

A requirement of GMP is that there be a suitable system for receiving and responding to product complaints. These include claims of lack of efficacy, adverse reactions or concerns about product

packaging and presentation. The APVMA requires that all adverse experiences are documented and reported[142].

For the anthrax vaccine currently sold by Fort Dodge, complaints are handled in the same way as complaints about the company's other products. However, the consortium, having no other products, would need to establish an alternative mechanism.

Provided the vaccine is produced in accordance with GMP, it is reasonable to assume that complaints would not be a common occurrence. Very likely, either the manufacturing or quality assurance manager would be capable of investigating the validity of complaints and could contract appropriately experienced veterinarians (private or Government) to investigate adverse experiences as necessary.

However, a mechanism for initial receipt and investigation of complaints is expected to be required. This includes a contact telephone number printed on the vaccine label.

It is assumed this could be achieved with the support of NSW Farmers. No allowance has been made for the cost of this.

10.14 Marketing

Assuming the continued involvement of rural merchandise distributors in the supply of vaccine to livestock owners, there would be no requirement for significant marketing simply as a result of the vaccine being produced at EMAI. No marketing costs have been allowed for.

Marketing may nonetheless be considered necessary to induce non-users of the vaccine to commence using it, on the basis that it was available at a lower price or because it would help to preserve market access.

This would be a classical marketing task involving the creation of awareness and relevance followed by altered attitudes and behaviour. A close analogy is the introduction of a significant variant of an existing animal health product.

Limiting the task to anthrax endemic areas of Victoria and NSW would make it easier and more economical than introducing a product used throughout Australia. Nonetheless, a sustained promotional campaign would be needed to overcome the perception that there was nothing new to say about anthrax.

The cost could vary greatly depending on its intensity and the methods used. An estimate of a modest campaign is shown in the following table:

[142] Adverse Experience Reporting Program, Australian Pesticides and Veterinary Medicines Authority

Item	Approximate Cost	Details
Press Advertising		
Production	$ 4,000	Includes design and layout
The Land	$ 20,000	12x3col @ $21.50pcm, 26 inserts
Weekly Times	$ 27,000	12x3col @ $29.04pcm, 26 inserts
Radio Advertising		
Production	$ 5,000	
NSW	$ 15,000	5 stations, 50 spots each, $60/spot
Victoria	$ 24,000	8 stations, 50 spots each, $60/spot
Direct Mail		
Brochure/leaflet production	$ 10,000	Includes design and print
Mailing	$ 10,000	10,000 recipients in anthrax areas
Total	$ 115,000	

11 Financial Assessment of the EMAI Option

Summary

The total cost of establishing a multi-purpose facility to produce anthrax vaccine at EMAI is estimated to be $2.6 million, comprising plant costs of $1.6m and commissioning costs of $0.9m. This includes provision for a vaccine bank of one year's supply.

The capital cost is consistent with three independent estimates from vaccine manufacturers, each of which has recent experience at establishing small vaccine manufacturing plants.

The cost of producing 450,000 doses of anthrax vaccine per year in the plant is estimated to be $0.88 - $1.76 per cattle dose, including all costs other than cost of capital. This makes no allowance for any batch failures. There is potential for this cost to be significantly higher, particularly initially, unless production efficiency and batch success are optimised.

If the spore count is reduced by two-thirds, making it similar to the Fort Dodge product, the dose cost is reduced to $0.75 to $1.10.

Major recurring costs include product liability insurance and depreciation, which in total add over 40 cents per dose.

More than two years would be required from commencement to availability of legally saleable vaccine.

Options for funding include livestock industry levies, stakeholder cost sharing and government. Unless funded by current levy recipients (eg MLA or AWI), levy funding would require legislation. A commercial partner is a possibility if the vaccine was sold on commercial terms and the facility could be used for other purposes.

11.1 Establishment Costs

11.1.1 Total Establishment Costs

The total costs of establishing a facility to produce anthrax vaccine at the required volume at EMAI is estimated to be $2.6 million. This includes $150,000 for modifications to one of the animal houses to allow potency testing, if required, and $400,000 to create a vaccine bank of one year's supply.

A facility capable of producing other products as well would cost an extra $180,000 approximately, not including equipment. The total cost would be dependent on the type of product and its manufacturing requirements and could vary quite significantly.

Construction and commissioning costs are shown in the following table. Subsequent sections provide further detail.

Summary of Capital Costs

Item	Cost	Description	Detail
Constructing and equipping manufacturing facility	1,572,000		Table 11.1
Plant commissioning	887,000		Table 11.2
Upgrade animal facility to PC3 for potency testing	150,000	Facility complying with AS, equipped with animal cages, two-way autoclave and access to incinerator.	Not needed if potency testing is outsourced. See discussion.
Total	2,609,000		

11.1.2 Plant Costs

The cost of constructing and equipping an anthrax vaccine facility that complies with prevailing standards and regulations was estimated in the report by Claxton (2002). This report systematically and meticulously assessed the capital cost of a facility that could produce between 300,000 and 1,000,000 doses of vaccine each year together with the operating costs of vaccine production.

Claxton calculated that the facility would cost $1,345,200 (with 8% CPI adjustment now $1.45 million). However, the report emphasised that this should be considered the base cost as there may be additional costs associated with compliance with codes of Good Manufacturing Practice (GMP), building codes, Australian Standards and environmental requirements such as waste disposal.

We have undertaken an assessment of current costs based on Claxton's estimates, updated to 2005 with additional data where warranted.

We have also obtained three separate and independent cost estimates from vaccine manufacturers who had recently designed, built and commissioned small vaccine manufacturing plants[143]. These estimates ranged from $1.5 to $1.8 million to build a facility that would meet the current GMP requirements of the APVMA.

Our calculations and these estimates are generally consistent with Claxton's calculations.

Construction costs are generally based on a building industry reference publication[144].

The difference between a sole purpose and multi-purpose plant is not significant in terms of the building itself. However, depending on the nature of the other products produced, equipment and ancillary costs may be important.

This estimate assumes that anthrax vaccine could only be produced in an entirely segregated section of the facility to avoid difficulties with decontamination and GMP compliance.

[143] Fort Dodge, Intervet and Allied Animal Health.

[144] Rawlinsons Construction Cost Guide 2005. Rawlhouse Publishing, Perth WA

Table 11.1 Plant Construction and Equipment

Item		
Premises	**Sole Purpose**	**Multi Purpose**
Shed	180,000	360,000
Cool room (to hold bulk material)	5,000	
Cool room (to store bulk and finished vaccine)	20,000	
Paths and access areas	50,000	
Interior lining (freezer panel)	100,000	
Air conditioning and treatment	120,000	
Plans and permits	20,000	
Project management	49,500	
Total	544,500	724,500
Equipment		
Water filter (Milli-Q system)	20,000	
Balance (for raw materials)	2,500	
pH meter	1,500	
Stainless steel benches & stools	8,000	
Computer (SOPs, inventory etc)	10,000	
Steam (non-attended boiler)	40,000	
Refrigerator (for seed material)	1,500	
Glassware etc	20,000	
Total	103,500	
Laboratory		
Formalin decontamination pass through hatch	25,000	
Autoclave (two way, accommodating 200L carboys)	600,000	
Hot air oven (to sterilise glassware)	40,000	
Benches and stools	5,000	
Microscope	12,000	
Biohazard unit (2.6 m)	15,000	
Formalin fumigation equipment (to fumigate laboratory)	2,000	
Laboratory supplies (pipettes etc)	10,000	
Peristaltic pumps (deliver inoculum to culture vessels and to fill product)	5,000	
Incubator, walk-in (to incubate growth of organism)	5,000	
Special air conditioning (supply Class D air)	5,000	
Total	724,000	
Other Items		
Security	30,000	
Product filling, labelling	50,000	
QC testing equipment/rooms	120,000	
Total All Items	1,572,000	

11.1.3 Commissioning Costs

Before being permitted to sell vaccine produced in the plant, there are a number of one-off costs that would be incurred. These are identified in the following table.

Table 11.2 Commissioning and Other Start-up Costs

Item	Cost Estimate	Rationale
Stability test program: Involves storage for 36 months [$10,000], testing at 0, 3, 6, 12, 18, 24, 36 months [7 samplings, 3 batches, viable spore count, pH, appearance, microbial contamination]; potency testing and safety testing at initial and 24 month timepoints (2 x $30,000 + 2 x $8,000 – 3 concurrent tests adds labour only and same PC3 room hire cost).	106,000	Requirement of registration. Assumes potency tests contracted to AAHL ($76,000 of total). Stability required for a minimum of 3 batches. Test meets APVMA requirements to support 3 year shelf life.
Production of stability batches	261,000	Assumptions: 3 batches of 400 flasks packed in 100ml and 500ml units, 50% batch success rate (Table 11.8)
Registration (dossier preparation, registration fee, responses to APVMA questions, finalisation of printed labels, receipt of registration certificate)	15,000	Product must be registered in order to be sold.
Compliance with cGMP	35,000	Validation of processes and procedures, writing of manufacturing documents, site master plan, SOPs etc. Claxton estimate of $20,000 now insufficient to meet new cGMP.
Bacillus anthracis master seed (34F2) validation	70,000	Compliance with 9CFR (safety, potency and other tests). Includes MLD determination + vaccine potency test. Assumes contracted to AAHL (60,000 of total).
Vaccine bank (one year's supply)	400,000	Assumed in EMAI proposal
Total	887,000	

11.2 Operating Costs

11.2.1 Total Operating Costs

The cost of producing 450,000 doses of anthrax vaccine per year in the EMAI facility, as described, will be between 88 cents and $1.76 per cattle dose.

This is based on the assumptions previously described and includes all costs other than the cost of capital.

The production aspects of efficiency of yield and batch success rate are the main variables affecting cost. This calculation assumes no batch failures. However, with a combination of low yield and 50% batch failure, using 100 flask batch sizes, the dose cost could actually be more than $8.00.

It is entirely possible that a substantially higher cost might be incurred soon after plant commissioning. With production experience, yield and batch success will improve, leading to a reduction in dose costs to those shown here. Pack size has minimal effect on costs.

The elements of the cost calculation are summarised in the following table, based on 400 flask production, no batch failures and employed labour. Further details are provided in subsequent sections.

Total Operating Costs ($)

	Low yield, 100ml pack	High yield, 500 ml pack	
Production	495,630	98,891	Table 11.8
Formulation	56,436	56,409	Table 11.8
Annual recurring costs	139,393	139,393	Table 11.4
Insurance	100,000	100,000	
Total	791,459	394,693	
Cost per dose	1.76	0.88	
Farmer price	2.07	1.03	

Using a commercial vaccine manufacturer to produce and sell the vaccine in the EMAI facility under contract may avoid insurance costs, as previously discussed. If the manufacturer applied a gross margin of 40%, the cost per dose would be between $1.09 and $2.56.

Selling at this cost of production, as envisaged, would result in a higher price than the Fort Dodge product. This may result in Fort Dodge remaining in the market and lead to two vaccines on the market, significantly increasing security of supply.

11.2.2 Variable Production Costs

Production costs for a cattle dose of 1ml were estimated by Claxton in 2002 to range from $0.12 to $0.92. These calculations were based on the following cost assumptions:

- No loss of batches due to failure.
- Total employment cost for a manager of $70,000 and technical staff $40,000 per annum.
- Average labour cost of $200 per person per day.
- To conform to specifications, the initial spore count must be ≥15 x 10^6 spores per ml.

Claxton's breakdown of costs was very thorough and is still relevant, but a number of factors need to be reconsidered.

The introduction of a new and more demanding code of GMP by the APVMA has a direct impact on the estimates of both capital and production costs. The main effect is to increase quality assurance costs. Under the new code a QA officer will need to be available full time throughout the production cycle. This adds another head at a scientific manager level.

Loss of batches can be due to a variety of factors as described by Claxton (2002), but principally inadequate viable spore count. The yield of spores is dependent on a variety of nutritional and environmental factors, not all of which are entirely predictable or known. While it is possible to obtain yields in excess of 10^9 per ml in liquid medium[145] the reproducibility of high yields is low and even changes in brand of casein have been sufficient to adversely impact on yield[146].

Information concerning the experiences of Arthur Webster and CSL suggest that from 50% to 67% of batches of bulk antigen will fail[147]. This translates to a doubling or tripling in the cost of production of bulk antigen and a significant flow-on increase in cost per dose.

It is difficult to anticipate how much this batch failure rate may be improved, and it remains a significant risk to efficient production. Experience in production will help, provided there is continuity of personnel, materials and production to allow improvements to be evaluated. It may also be possible to obtain expertise from manufacturers in other countries. However, even in ideal situations, a finite proportion of batches will be lost.

Claxton reported that, if 25% of bulk antigen batches failed to produce an acceptable yield, "the average production cost per dose is likely to be 30-45 cents" (page 27). However, the report highlighted that "up to 50% of bulk antigens produced may have to be discarded because of low spore count" (page 27).

Claxton's calculations were based on three production sizes (either 100, 200 or 400 culture flasks in each batch) and two yields of viable spores (either 10^8 or $10^{8.7}$ per ml).

Updated calculations incorporating current costs are shown in the following table (Table 11.8). Our summary calculations are based on the 400-flask example, which provides the lowest dose cost.

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[145] Brewer et al 1946b.

[146] Sutton 1947.

[147] Peter Claxton (pers comm. 2005) and Kevin Ellard (pers comm. 2005)

Table 11.8: Production Costs

2005 COST CALCULATIONS						
Number of flasks	100	100	200	200	400	400
Yield per ml bulk antigen (spore count)	1.0E+08	5.0E+08	1.0E+08	5.0E+08	1.0E+08	5.0E+08
Bulk antigen volume (L)	1.5	1.5	3.0	3.0	6.0	6.0
Total bulk antigen (spore count)	1.5E+11	7.5E+11	3.0E+11	1.5E+12	6.0E+11	3.0E+12
Bulk antigen doses (@ 1.5*10^7 spores/dose)	10,000	50,119	20,000	100,237	40,000	200,475
Batch cost ($): TOTAL	**40,019**	**40,019**	**41,412**	**41,412**	**44,056**	**44,056**
Batch cost ($): labour	14,974	14,974	14,974	14,974	14,974	14,974
Batch cost ($): materials, power, gas, water, maintenance, clothes, environmental monitoring	9,506	9,506	10,899	10,899	13,543	13,543
Batch cost ($): cGMP QA/QC and mfg mgr	15,539	15,539	15,539	15,539	15,539	15,539
Cost per 150,000 doses ($)	600,285	119,773	310,590	61,971	165,210	32,964
Formulation Cost 150,000 doses (100ml pack) ($) TOTAL	**18,812**	**19,551**	**18,812**	**19,551**	**18,812**	**19,551**
Formulation cost ($): labour	4,633	4,633	4,633	4,633	4,633	4,633
Formulation cost ($): materials, packaging, power, gas, water, maintenance, clothes, environmental monitoring	8,999	9,738	8,999	9,738	8,999	9,738
Formulation cost ($): cGMP QA/QC and mfg mgr	5,180	5,180	5,180	5,180	5,180	5,180
Finished product cost (150,000 doses) ($)	619,097	139,324	329,402	81,522	184,022	52,515
Cost per 1ml dose (100ml pack) ($)	4.13	0.93	2.20	0.54	1.23	0.35
Formulation Cost 150,000 doses (500ml pack) ($) TOTAL	**18,064**	**18,803**	**18,064**	**18,803**	**18,064**	**18,803**
Formulation cost ($): labour	4,633	4,633	4,633	4,633	4,633	4,633
Formulation cost ($): materials, packaging, power, gas, water, maintenance, clothes, environmental monitoring	8,251	8,990	8,251	8,990	8,251	8,990
Formulation cost ($): cGMP QA/QC and mfg mgr	5,180	5,180	5,180	5,180	5,180	5,180
Finished product cost (150,000 doses) ($)	618,349	138,576	328,654	80,774	183,274	51,767
Cost per 1ml dose (500ml pack) ($)	4.12	0.92	2.19	0.54	1.22	0.35
Bulk antigen batch success rate	**1.00**					
Changes: 2005 compared with Claxton (2002)						
Labour: daily rates of pay increased						
Labour: number of days per stage increased (based on manufacturing methods of FAO 1991 and requirements of PC3 and cGMP)						
cGMP: increased burden of monitoring and documentation of every manufacturing step now required by GMP license						
Environmental monitoring: adds approximately $10,000 per campaign, required by cGMP and PC3 standard						

The cost of labour was calculated based on the following rates. These are somewhat higher than those used by Claxton but have been independently verified.

POSITION	Annual Salary	Super	Other on-costs	Annual cost	Casual load	Daily rate
Manufacturing Manager	85,000	7,650	18,530	111,180	20%	533.7
Quality (Assurance / Quality Control) Manager	80,000	7,200	17,440	104,640	20%	502.3
Technical Officers (up to 3 at various stages of production)	45,000	4,050	9,810	58,860	20%	282.5
Regulatory Affairs	55,000	4,950	11,990	71,940	20%	345.3

As shown in Table 11.8, labour costs have only been applied during the period of production and are not full-time. In the case of the Quality Manager, it is not certain this would meet the APVMA's preference for a full time QA manager.

11.2.3 Annual Recurring Costs

Claxton's report noted that annual recurring costs (regulatory costs [GMP audits, manufacturer license fee, AQIS permits], security, process validation) were not included in the production cost.

Other costs identified in the report as not included were insurance (pages 25-26), depreciation (page 26), waste disposal (page 21) and regulatory affairs (page 26).

Not all costs have risen. For example, potency testing (which was not included in the Claxton costing) of each batch is expected to be less onerous if it could be based on (viable) spore count rather than animal testing, as required by pharmacopoeial monographs. As discussed previously, this appears likely.

The most significant recurring costs are product liability insurance, depreciation and the APVMA product levy. In total, these add over 50 cents to the cost per dose.

The following table shows a full list of recurring costs including those mentioned by Claxton.

Table 11. 4 Annual Recurring Costs

Item	Cost	Detail
Regulatory Related		
Staff training	3,200	Time for preparation and delivery of training by Quality Manager.
Documentation review & reissue	2,600	Documentation revision demands imposed by 2005 cGMP.
APVMA Annual GMP inspection	1,000	Covers cost of audit by an accredited GMP auditor
APVMA Manufacturers Licence Fee	1,500	Fee for Category 1 facility (immunobiologicals). May be paid in 4 yearly instalments.
APVMA Product Levy	4,050	Levy 0.9% of sales
AQIS Approved Premises permit	1,000	
AQIS in vivo permits	680	
Preparation for APVMA audits – labour	3,200	
Preparation of AQIS Permit applications	600	
Regulatory Sub Total		17,830
Equipment Validations		
HEPA filters and Laminar Flow units	2,500	
Autoclave	1,500	
Sterilizing oven	500	
Other	500	
Air flow validation	1,500	Requirement of PC-3 facilities
Calibration of scales	500	GMP requirement
Security	5,000	Additional security anticipated when AG report issued.
Health monitoring of staff	1,000	Periodic health monitoring recommended by PC-3 requirements
Equipment replacement	1,000	Assumed each HEPA filter needs to be replaced every 5 years.
Storage		
Retention sample storage	1,200	Estimate: $3/day
Master seed storage (at two sites)	5,000	Estimate: $12/day
Equipment Maintenance		
Scheduled and as needed	5,000	
Depreciatiion		
Plant	13,613	Rate 2.5%
Equipment	82,750	Rate 10%
Total	139,393	

11.3 Funding The EMAI Option

Options for funding the EMAI option include levies, stakeholder cost sharing, a commercial partner and government.

11.3.1 Levies

In principle, livestock industry levy funds could be used to fund establishment of a vaccine facility at EMAI.

By means of legislation, a new levy or an increase in a current levy could be introduced, or the consortium could be authorised to receive funds from current levies. Alternatively, one or more of the current levy fund recipients such as MLA and AWI could contribute the funds without need for legislation.

There are four levies currently imposed on the livestock industry. Details are shown in the following table.

Levy	Description	Collection Legislation	Disbursement Legislation
Cattle and Livestock Transaction Levy	Payable on transactions involving cattle, sheep, lambs and goats. Used to fund research, development and marketing activities through Meat and Livestock Australia, animal health programs conducted by Animal Health Australia, plus contributions to animal health programs and the National Residue Survey.	*Primary Industries Levies and Charges Collection Act 1991* *Primary Industries (Excise) Levies Act 1999* *National Residue Survey (Excise) Levy Act 1998* *National Residue Survey (Customs) Levy Act 1998*	*Australian Meat and Live-stock Industry Act 1997* *Australian Animal Health Council (Live-stock Industries) Funding Act 1996*
Cattle and Livestock (Producers) Export Charge	Payable on cattle and livestock exported from Australia. Used to fund research, development and marketing activities through Meat and Livestock Australia, animal health programs through Animal Health Australia, plus contributions to the Brucellosis and Tuberculosis Eradication Council and the National Residue Survey.	*Primary Industries Levies and Charges Collection Act 1991* *Primary Industries (Customs) Charges Act 1999* *National Residue Survey (Customs) Levy Act 1998*	*Australian Meat and Live-stock Industry Act 1997* *Australian Animal Health Council (Live-stock Industries) Funding Act 1996*
Cattle and Livestock Exporters Charge	Payable on cattle and livestock exported from Australia. Used to fund research, development and marketing activities through LiveCorp.	*Primary Industries Levies and Charges Collection Act 1991* *Primary Industries (Customs) Charges Act 1999*	*Australian Meat and Live-stock Industry Act 1997*
Wool Levy and Export Charge	Payable on shorn wool sold or used in the production of other goods. Used to provide funding for research and development programs administered by Australian Wool Innovation.	*Primary Industries Levies and Charges Collection Act 1991* *Primary Industries (Customs) Charges Act 1999* *Primary Industries (Excise) Levies Act 1999*	*Wool Services Privatisation Act 2000*

Revenue collected from levies in 2002-03 and 2003-04 was as follows[148]:

	Collections 2002/2003 ($000)	Collections 2003/2004 ($000)
Cattle transaction (incl. export)	50,651	48,934
Live animal export (commenced 1 Mar 04)	-	615
Livestock transaction (incl. export)	31, 539	31,240
Wool	60,222	42,739

Relevant disbursements of levy funds in 2003-04 were:

	($000)
Animal Health Australia	7,899
Meat & Livestock Australia	
- Marketing	47,965
- Research	21,706
Australian Wool Innovation	42,739
National Residue Survey	5,866
National Cattle Disease Eradication	2

There are three ways in which levy funds could be used to fund the cost of a vaccine facility or underwrite vaccine production:

1. The introduction of an additional levy or an increase in a current levy (eg the Cattle and Livestock Transaction Levy) specifically for the purpose.

   The levy could be imposed for a limited period if it was simply to cover establishment costs.

   This option would require amendment to current levy legislation.

2. The vaccine production entity could be authorised to receive funds from current levies, either one-off or longer term.

   Current legislation only allows disbursement of levy funds to the designated industry marketing, research or export bodies. Thus amendment to the legislation would be required.

3. By one or more of the current recipients of the funds agreeing to contribute the funds.

   This would involve diversion of funds from other projects but no change to legislation.

In practical terms all three of these has the potential to be controversial. The response to the recent proposal to increase the cattle transaction levy to fund a higher level of market development and promotion activities is indicative of what may occur.

[148] Levies Revenue Service Report to Stakeholders 2003-04

As anthrax is a relatively low level sporadic problem and only occurs in certain areas of Victoria, NSW and Queensland, there may be objection to a levy on all producers.

For MLA and AWI, it is not clear whether there would be sufficient uncommitted funds to enable their diversion to this project. For example, the report of the Beef Industry Funding Steering Committee (April 2005) included an examination into alternatives to a levy increase for funding greater marketing development and promotion expenditure. It reported that a 2003 Cattle Council review of MLA programs and expenditure found:

"… on-farm R&D programs did not have significant surplus funds to be able to divert into marketing."

It also reported that the Cattle Council found there were no surplus funds in either the National Residue Survey allocation or industry reserves. (A copy of the relevant sections from the report is included in Appendix 3.)

Nonetheless, the level of funds needed for this project is substantially less than under consideration by the Steering Committee. It is obvious from the disbursement figures shown above that as a proportion of the funds received by MLA and AWI each year, they are reasonably minor.

It could be argued that, provided these organisations were given sufficient notice of the requirement for the funds so they were able to make necessary adjustments to their forward commitments, it would not be impractical for them to do so.

Whether that is a realistic option is a matter for the MLA and AWI boards together with the peak organisations and beyond the scope of this report.

11.3.2 Other Funding Options

Potential options for funding the vaccine production facility other than levies are stakeholder cost sharing, a commercial partner and government.

11.3.2.1 Stakeholder Cost Sharing

Each of the stakeholder organisations could contribute the funds on an agreed basis, potentially in exchange for equity in the project.

Precedent for this was created with the Exotic Animal Disease Response Agreement, managed by Animal Health Australia, which defines how the costs of control are to be shared in the event of an outbreak of certain diseases.

Under that agreement, anthrax is classified as a Category 3 disease, which means the costs of controlling a major outbreak are to be shared 50% by government and 50% by the applicable industries.

11.3.2.2 Commercial Partner

It may be that a commercial partner could be attracted to this project, providing some or all of the capital expenditure.

Options range from full ownership of the facility through to joint ownership with the consortium and, perhaps, a loan to the consortium.

Involvement would be more likely if the vaccine was sold on commercial terms rather than at cost and the land was provided at no cost as envisaged. It would also be more likely if the facility was multi-purpose and the partner was able to use it for producing other products.

The IWP has expressed concerns about the involvement of a commercial partner based on assumptions about future uncertainty, the potential for a change in business intentions and loss of

"control" by industry. However, most of these concerns could be adequately addressed by means of a contract.

11.3.2.3 Government Funding

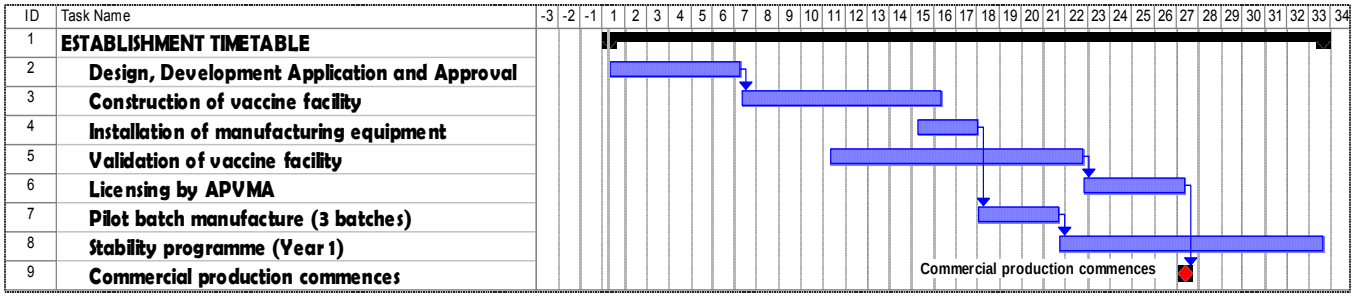
The NSW and Commonwealth governments could fund the project, although both have ruled this out.

There may be greater justification for government funding if a key rationale for establishing the facility is to enhance the country's ability to respond to bio-terrorism.

12 Establishment Timetable

Establishment of a facility at EMAI is considered likely to require at least two years before vaccine can be produced and legally used by livestock owners.

The main time elements are as follows:



13 Summary of Options

The available options for reducing the risk to the livestock industries from anthrax are summarised as follows:

Continue the international search for a second source of vaccine, on the basis that there are options that have not been investigated.

Wait for developments with human anthrax vaccines to produce an importable recombinant vaccine for livestock.

Establish a vaccine bank, using imported vaccine.

Reduce the risk of loss of market access due to anthrax by measures such as ensuring cattle are anthrax free and/or protected by vaccination prior to entry into feedlots.

Establish a vaccine plant at EMAI as envisaged.

Prepare contingency plan for establishment of a vaccine plant at EMAI, which is only triggered when the current source is disrupted and no resumption or alternative source appears likely.

13.1 Explore alternative second sources

The DAFF and IWP search for a second source of vaccine capable of being imported into Australia, undertaken several years ago, did not explore all the available options.

Moreover, assumptions were made about the likelihood of certain plants being able to comply with Australian quarantine and GMP requirements that may not have been valid, or may no be longer valid because of subsequent plant upgrades.

A more complete investigation remains an option prior to the commitment of significant funds on other alternatives.

Based on previous experience, investigating vaccine sources is frustratingly slow and may necessitate sending someone to undertake a physical inspection. Further, the chances of finding a product suitable for importation into Australia are probably relatively low given current sensitivity with respect to BSE and other exotic diseases.

Nonetheless, if a second source could be located on commercial terms, this would represent the greatest level of risk reduction for the least possible cost.

The Veterinary Laboratories Agency (VLA) is ceasing anthrax vaccine production for the UK and is actively seeking another source. Continued contact with VLA could be useful as the source it identifies may also prove satisfactory for Australia.

13.2 Wait for alternative technology

With the enormous level of resources now allocated to preventing anthrax in humans, particularly in the USA, an alternative to the Sterne vaccine for protecting livestock is expected to emerge within the foreseeable future.

Already a two dose recombinant vaccine based on protective antigen will soon be available for humans. Protective antigen is known to be similarly effective in livestock.

Even if the human vaccines under development require multiple doses in order to provide enduring immunity, only short-term immunity may be necessary for cattle (or sheep) in feedlots. It is possible

that rPA or other vaccine technologies may be quite suitable as single dose products for livestock in feedlots.

It is reasonable to expect that a recombinant anthrax vaccine produced in another country under GMP conditions using no animal components could be imported into Australia without undue difficulty, and that such a scenario may be feasible within five to ten years.

The principal uncertainty is the cost. Although recombinant vaccines tend to be inexpensive to produce, there may be research and development costs to recover that lead to a higher price than the current product. The price would decline as new products entered the market and patents expired.

13.3 A vaccine bank

As a means of achieving greater security of supply, a vaccine bank would be relatively effective. Indeed, it would provide greater security of supply than local manufacture alone, albeit without affecting the vaccine price.

The main uncertainty is the size of the bank, a function of the risk scenarios it is intended to address. These include outbreak scenarios and assumptions about the period needed to establish another source of supply if the existing one were to fail.

Given the assumed low prospect of success locating a second source of vaccine, as a practical reality a vaccine bank probably represents the greatest quantum of risk reduction for the lowest cost.

Depending on the thoroughness of the search for a second source, consideration may need to be given to whether a year's supply is sufficient.

13.4 Reduce market risk

Measures could be taken to reduce the risk of loss of market access due to anthrax, particularly for cattle feedlots.

Currently, cattle are vaccinated upon entering the feedlot. This leaves a period of up to two weeks in which the disease may occur prior to the development of immunity. As there is little prospect that cattle would be exposed to anthrax while in the feedlot, vaccination is unlikely to provide any disease control benefit.

If cattle were vaccinated at least two weeks prior to entering the feedlot, any incubating cases would occur prior to feedlot entry on the farm, confining the damage and avoiding any link to the feedlot.

This could be achieved by the feedlot industry introducing into its code of conduct an obligatory requirement for anthrax vaccination two weeks prior to feedlot entry.

Other markets considered particularly vulnerable to closure due to anthrax could be similarly identified and a targeted regime of vaccination introduced.

Alternatively, to satisfy the OIE recommendation, cattle could be quarantined for 20 days on farm or at a special holding facility, and only cattle from sources not affected by anthrax selected for feedlot entry. It may also be possible to diminish risk by persuading trading partners to observe the recommendations of OIE and accept imports arising from animals in establishments with no case of anthrax during the 20 days prior to slaughter.

13.5 A vaccine plant

Immediate Construction

The immediate construction of a plant at EMAI to produce vaccine remains an option if the capital cost is underwritten as envisaged.

A lower vaccine cost than the current product is a possibility, particularly over time, but appears unlikely in the short term.

Some of the risks associated with the current source of supply are removed or reduced, although some additional risk is introduced. If associated with a vaccine bank, as discussed, these are mostly removed.

As this option has never been motivated by market economics, beyond undertaking a realistic assessment of the costs involved there is little point in applying such analysis now. If those with responsibility for the required capital choose to commit to the construction of a vaccine plant, vaccine production can be achieved.

Delayed Construction

An alternative to immediate construction of a vaccine plant may be delayed construction, triggered by loss of the current source of supply.

Trigger events could include any kind of disruption to the current source accompanied by low prospects for short-term restoration or the establishment of an alternative.

This scenario would envisage detailed preparations to allow fast-track construction to be undertaken.

The detailed preparations could include:

- The import of both the master seed vaccine strain and the potency testing strain of *Bacillus anthracis* and maintenance in secure storage until required,
- Preparation of non-manufacturing sections of the APVMA regulatory dossier,
- Selected GMP documentation preparation, and
- Development of detailed plans for a manufacturing facility with the help of a “manufacturing advisory group” formed to ensure a rapid start.
- Execution of contracts required to secure EMAI site access and any other critical elements.

It would even be possible to involve a third party vaccine manufacturer on the basis that it would be responsible for producing the vaccine should it be necessary. This would almost certainly reduce the time required, albeit at higher cost.

13.6 Bio-terrorism Scenarios

If bio-terrorism were to become the driving force for vaccine supply, it is assumed that funding support from the Australian National Counter-Terrorism Plan may be available.

14 Conclusions

Whether the local production of anthrax vaccine is an appropriate response to the issues surrounding the disease is not only a question of cost and feasibility. It also raises issues as to whether the threat to trade can be reduced in other ways, whether anthrax is sufficiently significant relative to other diseases for which there may not be a vaccine produced locally, and whether the bioterrorism scenarios are valid. These are matters of judgement with limited objective information to provide assistance.

If it is considered that Australia requires greater security of supply of anthrax vaccine, employing a precautionary approach, our investigations suggest this would not be achieved by local production alone.

Such security could be achieved with a second source of supply or a vaccine bank, both at far lower cost than local production. A vaccine bank combined with local production would also increase security of supply.

A vaccine bank combined with a contingency plan to build a vaccine plant if there is a serious disruption to supply, would achieve virtually the same result provided the bank held enough stock to cover the period before production commenced.

If it is considered that Australia requires a more economical anthrax vaccine, our investigations indicate this would not be achieved by local production, at least within the assumptions that we have applied. A greater level of subsidy than envisaged would be necessary for this to occur. A second international source of supply, if obtainable, is probably more likely to contribute to a lower price.

If local manufacture were to proceed, we can confirm that although the challenges should not be underestimated, there are no insurmountable barriers to establishing a plant at EMAI to produce anthrax vaccine.

However, given the rapid developments in human anthrax vaccine development now occurring, it may be that the plant would be redundant within ten to fifteen years.

15 Appendices

Appendix 1

John McGarvie Smith biography

Appendix 2

Primary Industries Ministerial Council resolutions
4.8 2 Oct 2003
5.16 19 May 2004

Appendix 3

Extract from report of Beef Industry Funding Steering Committee (April 2005)

Appendix 4

Bibliography

Appendix 5

Manual for the production of anthrax vaccine FAO Animal Production and Health Paper 87; Dr R.P. Misra (1991)

Appendix 6

Colorado Serum Company product list

Appendices

Appendix 1

John McGarvie Smith

John McGarvie Smith was born at Sydney in 1844. At 13 years of age he had to make his own living, and having learned the trade of watchmaker and jeweller, opened a business for himself at Sydney in 1866. He carried on this business for about 20 years. He took up photography, which led to his studying chemistry at the university of Sydney about 1867, and later, metallurgy. He set up as an assayer and metallurgist about the year 1888. He developed improvements in the treatment of refractory ores and his advice was of great value in dealing with problems of this kind at the Sunny Corner mining-field and at Broken Hill. At Mount Morgan, Queensland, he did important work in connexion with the chlorine process of extracting gold.

He took up the study of bacteriology, and did a large amount of research endeavouring to find a vaccine against the effects of snake bite. He collected a large number of venomous snakes which he handled himself when extracting their venom. He eventually came to the conclusion that it was bacteriologically impossible to inoculate against snake-bite, but while carrying out his investigations he collected a large amount of information about the relative virulence of the venom of Australian snakes.

His most important research was in connexion with anthrax. Pasteur had discovered a vaccine, which, however, would not keep, and Smith after long experimenting found an effective vaccine which would keep for an indefinite period. This he treated as a business secret for many years, but a few months before his death he handed the formula to representatives of the government of New South Wales. He also gave £10,000 to endow a McGarvie Smith Institute. While making his investigations Smith travelled extensively in Europe and the United States and visited many laboratories. He was a man of great determination and remarkable personality. All his life he had a passion for work, but he spared time in his youth to become a good rifle shot. He married the widow of D. H. Deniehy (q.v.) who died many years before his own death at Sydney on 6 September 1918. He had no children.

The Sydney Morning Herald, 7 September 1918; W. S. Dun, *Journal and Proceedings Royal Society of New South Wales*, vol. LIII, p. 11; *Industrial Australian and Mining Standard*, 12 September 1918; *Sydney Directories*, 1867, 1885, 1889.

Appendix 2

Primary Industries Ministerial Council	MEETING NUMBER: 5
	LOCATION: ADELAIDE
	DATE: 19 MAY 2004
RESOLUTION	No: 5.16

ANTHRAX VACCINE – ENSURING A RELIABLE SUPPLY

1. Anthrax vaccine has not been manufactured in Australia since 1997. US-manufactured vaccine is imported by Fort Dodge Australia Pty Ltd, is registered and is readily available as required in Australia.

2. The grazing industries and governments are concerned to ensure a reliable supply of anthrax vaccine to manage the risks of a major outbreak or interruption to the supply. Official advice is that the risk of terrorism involving anthrax is low. There is some concern that outbreaks of anthrax may affect market access for live animals and product, particularly in some sensitive Asian markets.

3. Industry has had carriage of pursuing a reliable supply of anthrax vaccine since 1999. Several avenues have been investigated that have not been viable. Since 2002, an Industry Working Group (IWG) has been negotiating with NSW Agriculture to fund and manage a facility to manufacture anthrax vaccine on NSW government land at the Elizabeth McArthur Agricultural Institute (EMAI). A report is being prepared on indemnity issues. If the outcome of this report is favourable, the IWG may once again request Meat and Livestock Australia (MLA) to develop a business plan.

4. In 2003, Animal Health Committee concluded that current supply arrangements were adequate to meet foreseeable demand but a national stockpile is a viable option to manage contingencies, such as the 1997 unusual anthrax incident in Victoria when 80,000 cattle doses were used within a few months. NSW and Victoria each hold reserves of 20-25,000 cattle doses and individual feedlot operators hold substantial reserve stocks to manage their own risk.

5. PIMC 4 (October 2003) asked Animal Health Australia to investigate and report on how the prophylactic use of anthrax vaccination for flocks and herds in higher risk areas can be encouraged and how a reliable supply of anthrax vaccine to meet any realistic foreseeable need can be assured.

6. Because of the very low risk of occurrence, it is not cost-effective to vaccinate on properties where anthrax has not previously occurred, except possibly if high value animals are at risk or if the consequential loss associated with an outbreak is high (e.g. cattle feedlots).

7. Some overseas customers for livestock and livestock products have reacted to cases of anthrax, especially if feedlots are involved, occasionally causing market disruption.

If cases are not well managed in a low-key way, some sensitive export markets may be disrupted.

8. Early reporting and investigation of suspect cases, quarantine, safe disposal of carcasses (by burning if possible) and mandatory vaccination of in-contact animals is successful in preventing most outbreaks. Animal health authorities in NSW and Victoria consider that mandatory, large-scale, annual vaccination is unwarranted and would not prevent all sporadic cases. Tangible, market-access or public good benefits to the whole industry of preventing the occurrence of sporadic cases have not been demonstrated.

9. Current estimated annual use of vaccine is approximately 300,000 to 400,000 cattle doses of which about half would be used in cattle feedlots and over 40% for live animal exports. The rest is used on properties in NSW and Victoria in recognised anthrax areas where sporadic cases occur most years.

10. Greater reliability of supply of anthrax vaccine could be assured by either a local manufacturing facility or a vaccine stockpile held in Australia if it is deemed necessary.

11. One years' normal use (300,000 cattle equivalent doses) would be an appropriate reserve of vaccine to address any realistic foreseeable risk. This would allow alternative arrangements in the unlikely situation that supply is interrupted.

12. Whether or not local manufacture is viable, a vaccine reserve in the interim would secure the supply of vaccine to meet any realistic, foreseeable risk while a business plan is being developed and, if warranted, a plant constructed.

RESOLUTIONS

13. Council:

(a) **NOTED** that industry market access can be disrupted in major outbreaks and in sporadic cases that are not well managed;

(b) **NOTED** it is only on properties on which anthrax has previously occurred that it would be cost-effective to vaccinate unless animals were of very high value or the consequential losses from quarantine or withholding periods were high;

(c) **NOTED** that promotion of prophylactic anthrax vaccination on properties other than those that have had a previous case cannot be justified unless access to overseas markets is at risk. Any promotion must avoid alarming customers or the public; and

(d) **REQUESTED** Animal Health Australia to investigate possible arrangements between relevant industry and government parties to fund an interim anthrax vaccine reserve to secure supply and negotiate a contract with the commercial supplier pending final determination on local manufacture.

Primary Industries Ministerial Council	MEETING NUMBER: 4
	LOCATION: PERTH
	DATE: 2 OCTOBER 2003
RESOLUTION	**No: 4.8**

ANTHRAX VACCINE - ENSURING A RELIABLE SUPPLY FOR AUSTRALIA'S FORESEEABLE NEEDS

1. Anthrax is normally a sporadic occurrence in Australia. Most cases occur in NSW, and to a lesser extent in Victoria, although there have also been a few cases in Queensland since 1993. An unusually large outbreak of anthrax occurred in northern Victoria in early 1997. A shortage of vaccine occurred due to the unexpected increase in demand, with around 80,000 doses being used.

2. Animals vaccinated twice at a 6–12 month interval are normally immune for life. The interim AUSVETPLAN disease strategy for anthrax recommends that vaccination should be compulsory for three years on infected premises.

3. Annual vaccination is precautionary to minimize the risk of further cases, although possibly unnecessary for many animals. It is important that vaccination is undertaken on properties where cases occur for at least the minimum three years. Vaccination annually may be warranted on some properties with a persistent history of cases. However, it is at least as important for carcases from anthrax cases to be disposed of safely, by burning if at all possible.

4. Fort Dodge Australia Ltd (Fort Dodge) currently imports anthrax vaccine from Colorado Serum Company, Denver, Co., USA which is registered for use in Australia by the Australian Pesticides and Veterinary Medicines Authority.

5. Industry has been endeavouring to procure an alternative, cheaper source of anthrax vaccine than the commercial product imported by Fort Dodge since the unusual outbreak of anthrax in Victoria in 1997, which utilised around 80,000 doses over three months.

6. In November 2002, the Industry Working Group (IWG) submitted a proposal to establish a manufacturing plant at the NSW Agriculture Elizabeth Macarthur Agricultural Institute, Camden (EMAI). The justification for local manufacture was the risk of market loss from anthrax outbreaks, the threat of bioterrorism and market failure, particularly if the US government banned the export of vaccine in order to satisfy increased domestic demand.

7. Animal Health Committee established a working party in November 2002 to examine and report on the current risk of an increase in anthrax cases particularly associated with the drought.

8. Animal Health Australia (AHA) was requested by Safemeat in December 2002 to prepare a report that would investigate a range of issues surrounding the proposal to establish an anthrax vaccine manufacturing facility in Australia. A Final Report was issued on 5 June 2003 and identified a number of risk issues with the proposal to establish a manufacturing facility at the EMAI.

9. Anthrax vaccine for animal use utilises the live naturally avirulent Sterne strain of *Bacillus anthracis* and, being a live vaccine, is unsuitable for use in humans. Health authorities in Australia have access to overseas manufactured vaccines for prophylactic use in high occupational risk groups (e.g. laboratory workers and military personnel). Because different vaccines are needed, there are no identifiable efficiencies in a shared approach with health authorities to help underpin security of vaccine supply.

10. Anthrax vaccine for animal use may be needed for four potential situations:
    (a) emergency vaccination – to prevent further cases when anthrax occur;
    (b) preventive (prophylactic) vaccination - to protect livestock on farms where anthrax has occurred in previous years;
    (c) for commercial purposes – some feedlots have policies to vaccinate all cattle for anthrax. Also, some countries require anthrax vaccination of sheep and/or cattle being imported from Australia; and
    (d) to respond to bioterrorism.

11. The principal objective of anthrax vaccine policy should be to ensure that there is a reliable supply of vaccine available to handle all foreseeable and realistic demands. This is important to ensure that vaccination can be undertaken on affected and at risk herds and flocks when cases occur (in conjunction with safe disposal of infected carcases by burning if possible), for prophylactic vaccination in high risk herds and flocks and for commercial export requirements.

12. Council noted advice that it is not anticipated the current drought will lead to a significant increase in the numbers of anthrax cases, although a small increase may occur in recognized areas when the drought breaks, and that agriterrorism involving the anthrax agent directed against animal industries in Australia is not a high risk at this time.

13. The stocks held by NSW and Victoria, some feedlots, the normal stock-on-hand with Fort Dodge and the expanded production capacity of Colorado Serum Company should be able to meet any likely emergency response needs. The feedlot industry has indicated that members holding reserves of anthrax vaccine would not be willing to lend it to other users.

14. The IWG continues to explore the establishment of an anthrax vaccine manufacturing facility in Australia, the current proposal being at EMAI. Their proposal on this to the NSW and Australian Governments in November 2002 was based on assumptions that the governments would agree to carry certain operational and product liability risks. Meat and Livestock Australia was to develop a business plan for this venture but this

has not been progressed. Despite this, the IWG is continuing to canvas support from the governments.

15. Council noted that a report would be provided out-of-session on how best to encourage prophylactic use of anthrax vaccine and how best to ensure a reliable anthrax vaccine supply. It was agreed that this report from AHA should assess cost-effectiveness and should take into account the outcomes of a NSW study on viability of a manufacturing facility.

RESOLUTIONS

16. Council:

(a) **NOTED** that the Animal Health Committee process to examine changed demand for anthrax vaccine in response to a range of variables, including the end of drought conditions, has not identified any likely significant increase in demand;

(b) **NOTED** that, at this time, the biosecurity risk from the malicious use of the anthrax agent directed at animal populations is not considered high, but that circumstances could change rapidly and therefore warrant on-going review by responsible agencies;

(c) **NOTED** the industry activities with respect to the establishment of a facility to manufacture anthrax vaccine for animal use in Australia;

(d) **NOTED** the merit in encouraging the prophylactic use of anthrax vaccination for flocks and herds in higher risk areas;

(e) **NOTED** that all options for ensuring a reliable supply of anthrax vaccine should be investigated;

(f) **AGREED** that industry, through Animal Health Australia with technical input from the Animal Health Committee, investigate and report out-of-session on how:
(i) the prophylactic use of anthrax vaccination for flocks and herds in higher risk areas can be encouraged; and
(ii) a reliable supply of anthrax vaccine to meet any realistic foreseeable need can be assured, including via a vaccine stockpile held in Australia; and

(g) **AGREED** that the terms of reference of the report undertaken by Animal Health Australia include cost effectiveness and consideration of the outcomes of the NSW consultancy.

Appendix 3

Extract from report of Beef Industry Funding Steering Committee (April 2005)

d. What are the possible sources of funds?

The committee considered several possible sources for these additional funds, as outlined in the table below.

Options	Consideration
1. Cut back in other areas of MLA marketing activities	• A 2003 Cattle Council review of all of MLA programs and expenditure found that there was very little opportunity to trim existing marketing programs, or divert funds from other areas of MLA. In fact, the CCA review expressed concern that there was insufficient funding available to undertake the marketing work currently required of MLA.
2. Move some of MLA's R&D funds into marketing	• The CCA review also found that with the transfer in 2002 of 20c from the National Residue Funding levy into MLA's R&D programs there was sufficient funds in R&D under the current work programs. It also acknowledged that the on-farm R&D programs did not have significant surplus funds to be able to divert into marketing. • It is expected that the 20c grainfed component will be reallocated back to NRS in 2007 due to the rundown of NRS reserves. • ALFA reviews the split between R&D and marketing annually and adjusts accordingly to industry priority. The current grainfed levy does not provide sufficient funds to support the recommended programs in this report.
3. Move funds from the animal health and National Residue Survey areas into marketing	• CCA has reviewed current allocations to AHA and NRS and found them to be necessary at current levels. • A special one-off transfer of $2m was made from grassfed cattle AHA levies into MLA's export program in 2004-05. • In 2005, a reallocation of the AHA levy to MLA was made by ALFA due to increases in AHA reserves. This is insufficient to support the programs recommended in this report.
4. Use funds from MLA and industry reserves	• MLA funds and reserves are required to be managed and retained by species. They cannot be transferred between species. • MLA cattle reserves, particularly grassfed, are already low, representing an average of three months income. • Industry reserves held by the Red Meat Advisory Council (RMAC) are required by law to be retained at present vales over time and are only available for emergency purposes. If used, they are required to be repaid.
5. Cut MLA's administrative and overhead costs	• MLA's corporate costs represent only 5.3% of revenue, and are low in comparison to similar organisations. This leaves 94.7% of revenue being spent on developing and implementing MLA's programs. • The CCA review again found that the costs relating to the management of MLA marketing programs was efficient and justifiable.

Appendix 4

Bibliography

CITATION	ABSTRACT
Aarabi I, Sotoodehnia A. (1984). The immunity conferred by anthrax avirulent uncapsulated live vaccine following different methods (intradermal and subcutaneous) of vaccination. Archives de l'Institut Razi.. 34/35: 45-49.	A trial of anthrax spore vaccine (prepared from an Iranian strain of B. anthracis by Sterne's method) on 57 sheep and 57 goats showed that subcutaneous inoculation was easier and gave better protection from challenge infection than intradermal inoculation. Vaccine prepared from unencapsulated local Iraqi strain C5. Saponin (0.1% MT) added. One dose contained 4-5 million spores. Sheep and goats were either vaccinated (ID or SC) or unvaccinated and challenged at up to 13 months after vaccination with 200MLD (vaccinates) or 1 MLD (controls) of virulent strain C2. All control animals died while at least 9/10 vaccinates survived in the SC groups. Sterne-like vaccine provided at least 13 months protection in 90% of vaccinates.
Abalakin VA. (1989). Role of humoral and cellular factors in the formation of postvaccinal immunity to anthrax in mice. Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii.. 5, 108-109.	Cellular immunity accounted for about 30% of protection against anthrax in mice immunized with B. anthracis protective antigen mixed with heat-inactivated vegetative cells of the bacillus, while humoral antitoxic immunity accounted for the remaining 70%. Other experiments with guinea pigs have demonstrated the existence of a somatic protective antigen, involved in cellular immunity.
Abdrakhmanov T. (1984). Indirect haemagglutination test for studying antibody formation in animals vaccinated against anthrax. Vestnik Sel'skokhozyaistvennoi Nauki Kazakhstana.. 5, 66-67.	Secondary Journal Source: Referativnyi Zhurnal, 82. Veterinariya 1984 No.10 abst. 10.82.89.
Abraham EP, Chain E, Fletcher CM, Gardner AD, Heatley NG, Jennings MA, Florey HW (1941). Further observations on penicillin. Lancet 238(6155): 177-189	One strain of *Bacillus anthracis* examined and as sensitive to inhibition by penicillin as Streps and Staphs.
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Adone R, Pasquali P, Rosa G la; Marianelli C, Muscillo M, Fasanella A, Francia M, Ciuchini F. (2002). Sequence analysis of the genes encoding for the major virulence factors of *Bacillus anthracis* vaccine strain 'Carbosap'. Journal of Applied Microbiology. . 93: 1, 117-121.	Aims: This study was performed to analyse the molecular characteristics of genes encoding for the major virulence factors in *Bacillus anthracis* vaccine strain 'Carbosap' compared with the wild B. anthracis strain, to evaluate the basis of attenuation. Methods and Results: The molecular characteristics of the B. anthracis 'Carbosap' vaccine strain, used as vaccine in Italy, were analysed in comparison with a B. anthracis virulent strain. Despite the presence of the two virulence plasmids pXO1 and pXO2, the 'Carbosap' strain proved to be protective for cattle. The presence of the regulatory genes atxA and pagR and the gerX operon, known to be involved in the virulence, was verified. In addition, all genes were sequenced. The results showed that no molecular differences between 'Carbosap' and the virulent strain were evident. Conclusions: The results of this study indicate that the attenuation of the 'Carbosap' vaccine strain is not due to the lack of virulence genes or to modifications occurring on the sequence of these genes. Therefore, other virulence factors, still unknown, could be involved in the pathogenic mechanisms. Significance and Impact of the Study: This paper adds new information regarding the molecular characteristics of the vaccine strain 'Carbosap' and highlights the need to better understand the virulence factors involved in the pathogenicity of B. anthracis strains.
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Agerso Y, Jensen LB, Givskov M, Roberts MC. (2002). The identification of a tetracycline resistance gene tet(M), on a Tn916-like transposon, in the Bacillus cereus group. FEMS Microbiol Lett. 214(2):251-6.	Danish Veterinary Institute, 1790 Copenhagen V, Denmark. In order to investigate whether resistance genes present in bacteria in manure could transfer to indigenous soil bacteria, resistant isolates belonging to the Bacillus cereus group (Bacillus cereus, *Bacillus anthracis* and Bacillus thuringiensis) were isolated from farm soil (72 isolates) and manure (12 isolates) samples. These isolates were screened for tetracycline resistance genes (tet(K), tet(L), tet(M), tet(O), tet(S) and tet(T)). Of 88 isolates examined, three (3.4%) isolates carried both tet(M) and tet(L) genes, while four (4.5%) isolates carried the tet(L) gene. Eighty-one (92.1%) isolates did not contain any of the tested genes. All tet(M) positive isolates carried transposon Tn916 and could transfer this mobile DNA element to other Gram-positive bacteria.
Ahmed AM, Babiker SEH, El-Sanosi SM. (1998). Effect of increasing the fermenter silicone circulation tubing system on production of anthrax live spore vaccine.	

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Al-Dabbass AH, Al-Abdali SM, Mahmoud AA, Muhamed MM. (1986). Immunizing activity of oil adjuvant attenuated spore vaccine of *Bacillus anthracis* in sheep. Journal of Veterinary Medicine, B 33: 5, 340-345.	Lanolin and liquid paraffin were used to make a new oil adjuvant anthrax vaccine, by using the well-known non-capsulated and avirulent anthrax strain 34F2. 18 mice, 30 guinea pigs, and 48 sheep were used in the experiment. The mice and guinea pigs were challenged with Pasteur strain II (JB17) and the sheep with a local virulent anthrax strain (Basrah). Most of the mice and guinea pigs resisted challenge 21 days after vaccination and all the sheep in the experiment resisted the challenge. In a field trial with 5000 sheep the vaccine gave protection against anthrax for two years as judged by the absence of the disease in any sheep during that time. No side effects or local reactions were seen at the site of injection. Laboratory and field study. Sheep received a 1ml dose of an experimental vaccine containing 10-20 million spores of Sterne 34F2 with water-in-oil (mixture of normal saline and lanolin and paraffin oil) adjuvant. Sheep challenged periodically over the next 24 months with a local Iraqi virulent strain. All vaccinated sheep survived the challenge. The field study followed 5000 vaccinated sheep for 2 years and observed no cases of anthrax. It was concluded that sheep were protected for at least 2 years. The relative contributions of the adjuvant or the high number of spores to the observed DOI is not known. The actual duration of immunity is not described but is presumably much longer than 2 years. No titration of spore numbers in the vaccine is described to determine the minimum necessary for a particular DOI
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Altboum Z, Gozes Y, Barnea A, Pass A, White M, Kobiler D. (2002). Postexposure prophylaxis against anthrax: evaluation of various treatment regimens in intranasally infected guinea pigs. Infect Immun. 70(11):6231-41.	Department of Infectious Diseases, Israel Institute for Biological Research, Ness-Ziona 74100, Israel. altboum@iibr.gov.il The efficiency of postexposure prophylaxis against *Bacillus anthracis* infection was tested in guinea pigs infected intranasally with either Vollum or strain ATCC 6605 spores (75 times the 50% lethal dose [LD(50)] and 87 times LD(50,) respectively). Starting 24 h postinfection, animals were treated three times per day for 14 days with ciprofloxacin, tetracycline, erythromycin, cefazolin, and trimethoprim-sulfamethoxazole (TMP-SMX). Administration of cefazolin and TMP-SMX failed to protect the animals, while ciprofloxacin, tetracycline, and erythromycin prevented death. Upon cessation of treatment all erythromycin-treated animals died; of the tetracycline-treated animals, two of eight infected with Vollum and one of nine infected with ATCC 6605 survived; and of the ciprofloxacin group injected with either 10 or 20 mg/kg of body weight, five of nine and five of five animals, respectively, survived. To test the added value of extending the treatment period, Vollum-infected (46 times the LD(50)) animals were treated for 30 days with ciprofloxacin or tetracycline, resulting in protection of eight of nine and nine of nine animals, respectively. Once treatment was discontinued, only four of eight and five of nine animals, respectively, survived. Following rechallenge (intramuscularly) of the survivors with 30 times the LD(50) of Vollum spores, all ciprofloxacin-treated animals were protected while none of the tetracycline-treated animals survived. In an attempt to confer protective immunity lasting beyond the termination of antibiotic administration, Vollum-infected animals were immunized with a protective antigen

	(PA)-based vaccine concurrently with treatment with either ciprofloxacin or tetracycline. The combined treatment protected eight of eight and nine of nine animals. Following cessation of antibiotic administration seven of eight and eight of eight animals survived, of which six of seven and eight of eight resisted rechallenge. These results indicate that a combined treatment of antibiotics together with a PA-based vaccine could provide long-term protection to prevent reoccurrence of anthrax disease.
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Ariel N, Zvi A, Makarova KS, Chitlaru T, Elhanany E, Velan B, Cohen S, Friedlander AM, Shafferman A. (2003). Genome-based bioinformatic selection of chromosomal *Bacillus anthracis* putative vaccine candidates coupled with proteomic identification of surface-associated antigens. Infection and Immunity. . 71: 8, 4563-4579.	*Bacillus anthracis* (Ames strain) chromosome-derived open reading frames (ORFs), predicted to code for surface exposed or virulence related proteins, were selected as B. anthracis-specific vaccine candidates by a multistep computational screen of the entire draft chromosome sequence (February 2001 version, 460 contigs, The Institute for Genomic Research, Rockville, Md.). The selection procedure combined preliminary annotation (sequence similarity searches and domain assignments), prediction of cellular localization, taxonomical and functional screen and additional filtering criteria (size, number of paralogs). The reductive strategy, combined with manual curation, resulted in selection of 240 candidate ORFs encoding proteins with putative known function, as well as 280 proteins of unknown function. Proteomic analysis of two-dimensional gels of a B. anthracis membrane fraction, verified the expression of some gene products. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry analyses allowed identification of 38 spots cross-reacting with sera from B. anthracis immunized animals. These spots were found to represent eight in vivo immunogens, comprising of EA1, Sap, and 6 proteins whose expression and immunogenicity was not reported before. Five of these 8 immunogens were preselected by the bioinformatic analysis (EA1, Sap, 2 novel SLH proteins and peroxiredoxin/AhpC), as vaccine candidates. This study demonstrates that a combination of the bioinformatic and proteomic strategies may be useful in promoting the development of next generation anthrax vaccine.
Arntzen C, Plotkin S, Dodet B (2005). Plant-derived vaccines and antibodies: potential and limitations. Vaccine 23: 1753-1756	
Artenstein AW, Opal SM, Cristofaro P, Palardy JE, Parejo NA, Green MD, Jhung JW. (2004). Chloroquine enhances survival in *Bacillus anthracis* intoxication. Journal of Infectious Diseases.. 190: 9, 1655-1660.	The intentional release of anthrax in the United States in 2001 resulted in 11 cases of inhalational disease, with an attendant mortality rate of 45%. Current therapeutic options for anthrax are limited; antimicrobials target only replicating organisms, thus allowing bacterial toxins to cause unchecked, devastating physiological derangements in the host. Novel approaches that target the cytotoxic effects of anthrax exotoxins are needed. Chloroquine (CQ), a commonly used antimalarial agent, endows anthrax-intoxicated murine peritoneal macrophages with a 50% and 35% marginal survival advantage at 2 and 4 h, respectively, over that of untreated control cells. The cell rescue is dose dependent and, at lower concentrations, results in delayed cell death. We subsequently studied the effect of CQ in BALB/c mice challenged with anthrax lethal toxin. CQ-treated mice demonstrated reduced tissue injury, as assessed by histopathological examination of the spleen and by peripheral blood differential cell count ratios. CQ significantly enhanced survival and may augment current treatment and prophylaxis options for this otherwise lethal infection.
Athamna A, Athamna M, Abu-Rashed N, Medlej B, Bast DJ, Rubinstein E. (2004b). Selection of *Bacillus anthracis* isolates resistant to antibiotics. J Antimicrob Chemother. 54(2):424-8.	The Triangle Research and Development Center, Kfar-Qaraa, Israel. OBJECTIVE: Long-term therapy for anthrax might induce antimicrobial resistance in *Bacillus anthracis*. The aim of the present study was to investigate the potential of 18 different antibiotics to select resistant isolates of B. anthracis, (ST-1 and Sterne strains). METHODS: Resistant isolates were selected by serial passages on brain heart infusion agar containing increasing concentrations of antibiotics (from the MIC upwards). RESULTS: The MICs of ciprofloxacin, ofloxacin and levofloxacin increased from 0.125-0.25 to 8 mg/L, that of moxifloxacin increased from 0.03-0.06 to 8 mg/L, in both strains, and the MIC of garenoxacin increased from 0.015 to 0.5 mg/L for the ST-1 strain and from 0.03 to 8 mg/L for the Sterne strain. The MICs of tetracycline and minocycline increased from 0.125 to 2-8 mg/L and 0.06 to 1

	mg/L, respectively. The MIC of vancomycin increased from 2.5 to 20 mg/L for the ST-1 strain and from 5 to 20 mg/L for the Sterne strain. Linezolid exhibited an MIC increase from 2 to 4 mg/L for both strains. The MIC of quinupristin/dalfopristin increased from 0.125 to 64-128 mg/L. Erythromycin demonstrated an MIC increase from 1 to 128 mg/L, that of clarithromycin increased from 0.125 to 8-64 mg/L and that of telithromycin increased from 0.06-0.125 to 1-4 mg/L. The clindamycin MIC increased from 0.125-0.25 to 8 mg/L. Penicillin G and amoxicillin MICs increased from <1 mg/L to 128-512 mg/L. Isolates made resistant to one fluoroquinolone exhibited cross-resistance to the other quinolones except the ST-1 mutant strain which remained susceptible to garenoxacin. Cross-resistance to fluoroquinolones did not correlate with resistance to other antibiotics. CONCLUSION: The ease with which B. anthracis can be made resistant in vitro suggests that close monitoring of patients treated for anthrax is mandatory.
Athamna A, Athamna M, Medlej B, Bast DJ, Rubinstein E. (2004c). In vitro post-antibiotic effect of fluoroquinolones, macrolides, beta-lactams, tetracyclines, vancomycin, clindamycin, linezolid, chloramphenicol, quinupristin/dalfopristin and rifampicin on *Bacillus anthracis*. J Antimicrob Chemother. 53(4):609-15.	The Triangle Research and Development Center, Kfar-Qaraa, Israel. OBJECTIVES: The aim of this study was to investigate in vitro the post-antibiotic effect (PAE) of 19 antibacterial agents against two strains of *Bacillus anthracis* (ST-1 and Sterne strains). METHODS: PAE was determined by calculating the time required for the viable counts of antibiotic-exposed bacteria (at concentrations of 10x MIC and exposure for 2 h) at 37 degrees C to increase by 1 log10 above the counts observed immediately after antibiotic removal compared with the corresponding time for controls not exposed to antibiotics. RESULTS: The PAEs of the fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin and garenoxacin) were 2-5 h. The macrolide (erythromycin, clarithromycin and telithromycin) PAEs were 1-4 h, and that of clindamycin was 2 h. The PAEs induced by tetracycline and minocycline were 1-3 h. The PAEs induced by the beta-lactams (penicillin G, amoxicillin and ceftriaxone), vancomycin, linezolid and chloramphenicol were 1-2 h. The PAE induced by rifampicin was 4-5 h. Quinupristin/dalfopristin had the longest PAE, lasting for 7-8 h. CONCLUSIONS: Our results indicate that the PAE is unrelated to the MIC but may be related to the rapidity of bacterial kill. These observations may bear importance on treatment regimens of human anthrax.
Athamna A, Athamna M, Nura A, Shlyakov E, Bast DJ, Farrell D, Rubinstein E. (2005). Is in vitro antibiotic combination more effective than single-drug therapy against anthrax? Antimicrob Agents Chemother. 49(4):1323-5.	Traingle Research and Development Center, Kfar-Qaraa, Tel Aviv University School of Medicine, Tel Aviv, Israel. Antibiotic combinations are used to enhance antibacterial efficacy and to prevent the development of resistance. We have tested a possible synergistic effect of several antibacterial combinations on *Bacillus anthracis*. The in vitro activities of antibiotic combinations against two strains of B. anthracis, strain Sterne and the Russian anthrax vaccine strain STi, were tested by the fractional inhibitory concentration (FIC) method, derived from the MICs of the agents in combination, and by measuring the rate of bacterial killing over time by several antibiotic combinations. The FIC results showed that synergism against both B. anthracis strains was observed only with the combination of rifampin and clindamycin. The telithromycin-amoxicillin combination showed synergism against strain Sterne only. All other combinations were either indifferent or antagonistic. The results of the bacterial time-kill study demonstrated indifferent effects for all combinations. These in vitro results demonstrate the difficulties in obtaining synergistic combinations of
Athamna A, Massalha M, Athamna M, Nura A, Medlej B, Ofek I, Bast D, Rubinstein E. (2004a). In vitro susceptibility of *Bacillus anthracis* to various antibacterial agents and their time-kill activity. J Antimicrob Chemother. 53(2): 247-51.	The Triangle Research And Development Center, Kfar-Qaraa, Israel. OBJECTIVES: To investigate the in vitro acquisition of resistance to antibiotics by *Bacillus anthracis*. METHODS: The in vitro activities of 18 antibacterial agents against two strains of B. anthracis, the Sterne strain and the Russian anthrax vaccine strain ST-1, were tested by determining the MICs and by measuring the rates of antibiotic kill at 5x and 10x MIC. RESULTS: The fluoroquinolones ciprofloxacin, ofloxacin, levofloxacin and moxifloxacin, the beta-lactams penicillin G and amoxicillin, the macrolide clarithromycin, the ketolide telithromycin, as well as clindamycin, rifampicin and quinupristin/dalfopristin had MICs in the range of 0.03-0.25 mg/L. Minocycline had an MIC of 0.03 mg/L, as did penicillin, against the ST-1 strain. Ciprofloxacin had an MIC of 0.03 mg/L against both strains. Erythromycin, vancomycin and the oxazolidinone linezolid were less active (MIC 0.5-2.5 mg/L). Ceftriaxone was the least active, having an MIC of 8.0 mg/L. Chloramphenicol was inactive (MIC > 256 mg/L). Quinupristin/dalfopristin, rifampicin and

	moxifloxacin showed the most rapid bacterial killing, achieving a complete eradication of detectable organisms (2 log(10) reduction within 0.5-3 h and 4 log(10) reduction within 0.5-4 h for both strains at concentrations of 5x and 10x the MIC). The beta-lactams and vancomycin demonstrated a 2-4 log(10) reduction within 5-15 h. Ceftriaxone had a similar effect to penicillin and amoxicillin against the ST-1 strain, but a slower effect than these two beta-lactams against the Sterne strain. The macrolides, tetracyclines and linezolid demonstrated a lower kill rate, while chloramphenicol did not kill at all. CONCLUSIONS: These data expand on the spectrum of agents recommended for the treatment of anthrax (ciprofloxacin, penicillin G and tetracyclines) and add new options, such as other fluoroquinolones, amoxicillin, rifampicin and quinupristin/dalfopristin, as potential therapeutic agents.
Atkinson WL, Pickering LK, Watson JC, Peter G. (2003). General immunization practices. In: Plotkin SA, Orenstein WA, ed. Vaccines, 4th ed. Philadelphia: Elsevier,:91-122.	
Atlas RM. (2001). Bioterrorism before and after September 11. Crit Rev Microbiol. 27 (4): 355–379.	[Methods of detection of anthrax – microscopy, FA staining, PCR etc]
Atlas RM. (2002). Bioterrorism: from threat to reality. Annual Review of Microbiology. 56: 167-185.	The fears and predictions of attacks with biological weapons, which were increasing at the close of the twentieth century, were transformed into reality not long after September 11, 2001, when several anthrax-laden letters were sent through the U.S. postal system. The attack challenged our medical preparedness and scientific understanding of the epidemiology of biothreat agents. It is fortunate that this was not a massive aerosol release that could have exposed hundreds of thousands. Rapid diagnoses and medical treatments limited casualties and increased survival rates, but tragically some individuals died of inhalational anthrax. Even as physicians tested new treatment regimes and scientists employed new ways of detecting anthrax and decontaminating the mail, new predictions were made for potentially even more devastating attacks with anthrax, smallpox, plague, tularemia, botulism, or hemorrhagic fever viruses. Fear gripped the nation. Law enforcement sought to find the villain(s) who sent the anthrax letters and to deter future bioterrorist attacks. The biomedical community began to seek new ways of protecting against such future threats of bioterrorism.
Auerbach S, Wright GG (1955). Studies on immunity in anthrax. VI. Immunizing activity of protective antigen against various strains of *Bacillus anthracis*. J Immunol 75: 129-133	[Turnbull 1991 #18]
Aulinger BA, Roehrl MH, Mekalanos JJ, Collier RJ, Wang JY. (2005). Combining anthrax vaccine and therapy: a dominant-negative inhibitor of anthrax toxin is also a potent and safe immunogen for vaccines. Infection and Immunity. 73: 6, 3408-3414.	Anthrax is caused by the unimpeded growth of *Bacillus anthracis* in the host and the secretion of toxins. The currently available vaccine is based on protective antigen (PA), a central component of anthrax toxin. Vaccination with PA raises no direct immune response against the bacilli and, being a natural toxin component, PA might be hazardous when used immediately following exposure to B. anthracis. Thus, we have sought to develop a vaccine or therapeutic agent that is safe and eliminates both secreted toxins and bacilli. To that end, we have previously developed a dually active vaccine by conjugating the capsular poly- gamma -D-glutamate (PGA) with PA to elicit the production of antibodies specific for both bacilli and toxins. In the present report, we describe the improved potency of anthrax vaccines through the use of a

	dominant-negative inhibitory (DNI) mutant to replace PA in PA or PA-PGA vaccines. When tested in mice, DNI alone is more immunogenic than PA, and DNI-PGA conjugate elicits significantly higher levels of antibodies against PA and PGA than PA-PGA conjugate. To explain the enhanced immunogenicity of DNI, we propose that the two point mutations in DNI may have improved epitopes of PA allowing better antigen presentation to helper T cells. Alternatively, these mutations may enhance the immunological processing of PA by altering endosomal trafficking of the toxin in antigen-presenting cells. Because DNI has previously been demonstrated to inhibit anthrax toxin, postexposure use of DNI-based vaccines, including conjugate vaccines, may provide improved immunogenicity and therapeutic activity simultaneously.
AVMA (2001). Anthrax backgrounder. American Veterinary Medicine Association. http://www.avma.org/pubhlth/biosecurity/anthrax_bgnd.pdf	
Aziz MA, Samer Singh Kumar PA, Rakesh Bhatnagar. (2002). Expression of protective antigen in transgenic plants: a step towards edible vaccine against anthrax. Biochemical and Biophysical Research Communications.. 299: 3, 345-351.	Protective antigen (PA) is the most potent molecule for vaccination against anthrax. In the present study, we have successfully integrated protective antigen gene in nuclear genome of tobacco plants by Agrobacterium mediated leaf-disc transformation method. Expression of protective antigen gene was detected by immunoblot analysis using antisera raised against purified PA. A distinct band of ~83 kDa lighted up in the protein extracted from transformed plants while there was no such band in untransformed plants. The plant expressed PA showed biological activity just like native PA, which was demonstrated by cytolytic assay on macrophage like cell lines with lethal factor. This study establishes for the first time expression of PA gene in a plant system and thus marks the first milestone towards developing edible vaccine against anthrax.
Baeumner AJ, Leonard B, McElwee J, Montagna RA. (2004). A rapid biosensor for viable B. anthracis spores. Analytical and Bioanalytical Chemistry. . 380: 1, 15-23.	A simple membrane-strip-based biosensor assay has been combined with a nucleic acid sequence-based amplification (NASBA) reaction for rapid (4 h) detection of a small number (ten) of viable B. anthracis spores. The biosensor is based on identification of a unique mRNA sequence from one of the anthrax toxin genes, the protective antigen (pag), encoded on the toxin plasmid, pXO1, and thus provides high specificity toward B. anthracis. Previously, the anthrax toxins activator (atxA) mRNA had been used in our laboratory for the development of a biosensor for the detection of a single B. anthracis spore within 12 h. Changing the target sequence to the pag mRNA provided the ability to shorten the overall assay time significantly. The vaccine strain of B. anthracis (Sterne strain) was used in all experiments. A 500-micro L sample containing as few as ten spores was mixed with 500 micro L growth medium and incubated for 30 min for spore germination and mRNA production. Thus, only spores that are viable were detected. Subsequently, RNA was extracted from lysed cells, selectively amplified using NASBA, and rapidly identified by the biosensor. While the biosensor assay requires only 15 min assay time, the overall process takes 4 h for detection of ten viable B. anthracis spores, and is shortened significantly if more spores are present. The biosensor is based on an oligonucleotide sandwich-hybridization assay format. It uses a membrane flow-through system with an immobilized DNA probe that hybridizes with the target sequence. Signal amplification is provided when the target sequence hybridizes to a second DNA probe that has been coupled to liposomes encapsulating the dye sulforhodamine B. The amount of liposomes captured in the detection zone can be read visually or quantified with a hand-held reflectometer. The biosensor can detect as little as 1 fmol target mRNA (1 nmol L-1). Specificity analysis revealed no cross-reactivity with 11 organisms tested, among them closely related species such as B. cereus, B. megaterium, B. subtilis, B. thuringiensis, Lactococcus lactis, Lactobacillus plantarum, and Chlostridium butyricum. Also, no false positive signals were obtained from nonviable

	spores. We suggest that this inexpensive biosensor is a viable option for rapid, on-site analysis providing highly specific data on the presence of viable B. anthracis spores.
Bailey WW. (1954). Antibiotic therapy in anthrax. J Amer Vet Med Assoc 124: 296	
Baillie L (1999). 3rd International Conference on Anthrax, University of Plymouth, 710 September 1998: preface. J Appl Microbiol 87: 187-188	
Baillie L, Fowler K, Turnbull PC. (1999). Human immune responses to the UK human anthrax vaccine. J Appl Microbiol. 87(2):306-8.	DERA Chemical and Biological Defence Sector, Porton Down, Salisbury, Wilts, UK. The IgG anti-protective antigen subclass antibody response of individuals who had been infected with anthrax was compared with that of healthy individuals immunized with the UK licensed anthrax vaccine. The predominant subclass in both groups was IgG1. In addition, IgG3 was seen in convalescent serum while vaccinees produced IgG2, IgG3 and IgG4 subclass. The significance of these results is discussed. Further work is required to determine the role of antibodies in mediating protective immunity in man.
Baillie L, Hebdon R, Flick-Smith H, Williamson D. (2003). Characterisation of the immune response to the UK human anthrax vaccine. FEMS Immunol Med Microbiol. 36(1-2):83-6.	Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 W Lombard Street, Baltimore, MD 21202, USA. baillie@umbi.umd.edu The UK human anthrax vaccine consists of the alum-precipitated culture supernatant of *Bacillus anthracis* Sterne. In addition to protective antigen (PA), the key immunogen, the vaccine also contains a number of other bacteria- and media-derived proteins. These proteins may contribute to the transient side effects experienced by some individuals and could influence the development of the PA-specific immune response. Bacterial cell-wall components have been shown to be potent immunomodulators. B. anthracis expresses two S-layer proteins, EA1 and Sap, which have been demonstrated to be immunogenic in animal studies. These are also immunogenic in man so that convalescent and post-immunisation sera contain specific antibodies to Ea1, and to a lesser extent, to Sap. To determine if these proteins are capable of modifying the protective immune response to PA, A/J mice were immunised with equivalent amounts of recombinant PA and S-layer proteins in the presence of alhydrogel. IgG isotype profiles were determined and the animals were subsequently challenged with spores of B. anthracis STI. The results suggest that there was no significant shift in IgG isotype profile and that the presence of the S-layer proteins did not adversely affect the protective immune response induced by PA.
Baillie L, Read TD. (2001). *Bacillus anthracis*, a bug with attitude! Current Opinion in Microbiology.. 4: 1, 78-81.	This review discusses the virulence factors of B. anthracis and current knowledge in B. anthracis-host interactions. A brief overview of the pathological aspects and clinical presentation of anthrax (cutaneous, pulmonary and gastrointestinal anthrax). Virulence factors discussed include lethal factor, oedema factor, protective antigen, bacterial capsule, plasmid-encoded virulence factors and chromosomal-encoded virulence factors.
Baillie L, Townend T, Walker N, Eriksson U, Williamson D. (2004). Characterization of the human immune response to the UK anthrax vaccine. FEMS Immunol Med Microbiol. 42(2):267-70.	Biodefense Vaccines, Biological Defense Research Directorate, Naval Medical Research Center, Silver Spring, MD 20910-7500, USA. bailliel@mmrc.navy.mil The anthrax bipartite lethal toxin (protective antigen (PA) and lethal factor (LF))-specific antibody responses of humans receiving the UK licensed anthrax vaccine were determined. The PA-specific IgG response peaked two weeks post immunization and fell back to pre-boost levels by week 12. The heterogeneity of the host population modulated the extent of the PA-specific antibody response. Significantly lower levels of LF-specific antibodies were also detected. Vaccinated individuals recognized the same PA epitope as the protective mouse lethal toxin neutralizing monoclonal 2D3 suggesting that this may also be a target for human protection.

Baillie L. (2001). The development of new vaccines against *Bacillus anthracis*. Journal of Applied Microbiology. . 91: 4, 609-613.	A discussion on B. anthracis, the aetiologic agent of anthrax is presented. The pathogenecity of the organism, in animals and man, virulence factors and measures to prevent anthrax are also discussed. Preventive measures include antimicrobials and vaccines. A brief account of the efforts being undertaken in the development of novel vaccines and UK-licensed human anthrax vaccines is also given.
Baillie LWJ, Fowler K, Turnbull PCB. (1999). Human immune responses to the UK human anthrax vaccine. J Appl Microbiol 87: 306-308	
Bakici MZ, Elaldi N, Bakir M, Dokmetas I, Erandac M, Turan M. (2002). Antimicrobial susceptibility of *Bacillus anthracis* in an endemic area. Scand J Infect Dis. 34(8):564-6.	Department of Clinical Microbiology, Faculty of Medicine, Cumhuriyet University, Sivas, Turkey. We aimed to test the antimicrobial susceptibility of 28 *Bacillus anthracis* strains isolated from cutaneous anthrax cases to various antimicrobial agents using the Sceptor automatic system in an anthrax endemic area. All strains tested were susceptible to penicillin (MIC < or = 0.03 microg/ml). Piperacillin-tazobactam and carbapenems showed good activity towards all strains. Trimethoprim-sulfamethoxazole and cefepime had no activity. Strains were also tested with other antimicrobials.
Bakulov IA, Gavrilov VA, Karakhanov AG. (1987). Erythrocytic diagnostic preparation for anthrax. Veterinariya, Moscow.. 7, 40-42.	Preparation of antigen for the indirect haemagglutination test was described. Cattle, sheep and pigs inoculated with "STI" anthrax vaccine developed antibody titres of 1:160-1:320, which persisted for 6-12 months. There was no advantage in injecting a second dose of vaccine 3 weeks after the first. Booster doses were required every 6 months.
Bakulov IA, Gavrilov VA, Kosyachenko NS. (1993). Current thoughts on safety of live vaccines against anthrax. Veterinariya (Moskva).. 1, 22-25.	
Bakulov IA, Gavrilov VA, Sobakin AS. (1993). Development of combined vaccine against anthrax and foot and mouth disease using monopreparations of new generation. Russian Agricultural Sciences.. 9, 12-20.	New combined vaccine was developed using avirulent *Bacillus anthracis* strain 55-VNIIVViM and inactivated cultural vaccine against A-22 and O-194 types of foot and mouth disease virus. Diethyl ethylene imine was used to inactivate the vaccine and aluminium hydroxide added for an adjuvant. Guineapigs immunized with combined vaccines showed formation of antibodies in complement fixation test and were protected against experimental infections in 100 and 85-90% respectively.
Bakulov IA, Gavrilov VA. (1991). Immunoprophylaxis of anthrax. Vestnik Sel'skokhozyaistvennoi Nauki (Moskva).. 8, 128-131.	Live lyophilised vaccine produced from *Bacillus anthracis* avirulent strain 55 sporulated organisms grown in liquid medium produced better immunity than vaccines grown on solid media. Laboratory results indicated that immunity against experimental challenge with different field strains lasted 1.5 years in cattle and sheep and 1 year in pigs. The vaccine remained immunogenic up to 2 years and was effective at half the dose of vaccines produced on solid media. No adverse effects were observed in some 400 million animals of different species immunized in several republics.
Bakulov IA, Selyaninov Yu O, Balyshev V M, Nikulin AN, Shchetnikova LA, Gorshkova TF. (2001). Development and investigation of the immunological properties of a mixed vaccine against anthrax and sheep's pox. Russian Agricultural Sciences.. 3, 56-61.	The developed experimental mixed vaccine against anthrax and sheep's pox is a harmless, nonreactive, and immunogenic preparation. It ensures the resistance of animals to infection by control reference strains of the pathogens of these diseases. The stress of the immunity induced by the preparation does not differ from that forming when commercial monovaccines are used. The biological activity of the two vaccine strains included in the mixed preparation is preserved at the initial level in the course of 12 months (the period of observation) when stored under the recommended conditions (4+or-2 degrees C).
Bales ME, Dannenberg AL, Brachman PS,	We used unpublished reports, published manuscripts, and communication with investigators to identify and summarize

Kaufmann AF, Klatsky PC, Ashford DA (2002). Epidemiologic Response to Anthrax Outbreaks: Field Investigations, 1950–2001. Emerg Infect Dis 8: 1163-1174	49 anthrax-related epidemiologic field investigations conducted by the Centers for Disease Control and Prevention from 1950 to August 2001. Of 41 investigations in which *Bacillus anthracis* caused human or animal disease, 24 were in agricultural settings, 11 in textile mills, and 6 in other settings. Among the other investigations, two focused on building decontamination, one was a response to bioterrorism threats, and five involved other causes. Knowledge gained in these investigations helped guide the public health response to the October 2001 intentional release of B. anthracis, especially by addressing the management of anthrax threats, prevention of occupational anthrax, use of antibiotic prophylaxis in exposed persons, use of vaccination, spread of B. anthracis spores in aerosols, clinical diagnostic and laboratory confirmation methods, techniques for environmental sampling of exposed surfaces, and methods for decontaminating buildings. 6 veterinarians with cutaneous anthrax acquired during necropsy of cattle.
Balogh K de; Bbalo GC, Bohm R, Chizyuka HGB, Kigan B, Komba GL, Muyoyeta PM, Tuchili LM, Turnbull PCB, de Vos V; Roberts DH, Fujikura T. (1994). Anthrax control and research, with special reference to national programme development in Africa: memorandum from a WHO meeting. Bulletin of the World Health Organization.. 72: 1, 13 22.	The prevalence of anthrax in both animal and human populations has been increasing in Africa. It was therefore appropriate for this WHO meeting to be convened in an endemic area of the Western Province of Zambia in 1992. The participants reviewed anthrax epidemiology and control in some African countries, elaborated national anthrax control and research programmes in Africa, discussed international cooperation and work plans, and elaborated recommendations for anthrax control in Africa. The discussions centred on anthrax surveillance and reporting systems, diagnosis, vaccine production and immunization, disinfection and decontamination, carcass disposal, treatment of human cases, health systems, as well as intersectoral cooperation between public health services, veterinary services and other services such as wildlife conservation, so that national control programmes could take full account of the conditions prevailing in epidemic situations in Africa. The recommendations are applicable in other regions where anthrax poses similar problems in public, animal and environmental health.
Ban J (2000). Agricultural biological warfare. An overview. Chemical and Biological Arms Control Institute. http://www.cbaci.org	STATE PROGRAMS: WWI Germany – anthrax and glanders. WWII FMD open air trials on cattle and reindeer on an island in Lake Peipus in NW Russia; potato beetle, turnip weevils, turnip bugs, antler moths, potatoe stalk rot; Iraq and camel pox (surrogate for small pox?), anti crop programs against rice, wheat, barley, maize, potatoes; other animal biologicals – rinderpest, African swine fever, vesicular stomatitis virus, contagious bovine pleuropneumonia, avian influenza, ORF, Newcastel disease. NON-STATE SABOTAGE: 4 reasons (no [immediate] human casualties; difficult to detect; easy to attack; easy dissemination of pathogens. Examples: mercury contamination of Israeli citrus exports; threat to contaminate Sri Lankan tea with cyanide; Chilean graps contaminated with cyanide; Mau Mau use of African milk bush toxin to poison cattle; fruit fly release in CA. Taiwan FMD outbreak in 1997 illustrates devastating effects of outbreak on trade. RECOMMENDATIONS: strategy, contingency plans, surveillance, diagnostics, laboratory support, training, cooperation, R&D, security assessment.
Barakat LA, Quentzel HL, Jernigan JA, et al. (2002). Fatal inhalational anthrax in a 94-year-old Connecticut woman. JAMA 287: 863–8.	[During et al 2001, 3]
Barnard JP, Friedlander AM. (1999). Vaccination against anthrax with attenuated recombinant strains of *Bacillus anthracis* that produce protective antigen. Infect Immun. 67(2):562-7.	Division of Bacteriology, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-5011, USA. The protective efficacy of several live, recombinant anthrax vaccines given in a single-dose regimen was assessed with Hartley guinea pigs. These live vaccines were created by transforming DeltaANR and DeltaSterne, two nonencapsulated, nontoxinogenic strains of *Bacillus anthracis*, with four different recombinant plasmids that express the anthrax protective antigen (PA) protein to various degrees. This enabled us to assess the effect of the chromosomal

	background of the strain, as well as the amount of PA produced, on protective efficacy. There were no significant strain-related effects on PA production in vitro, plasmid stability in vivo, survival of the immunizing strain in the host, or protective efficacy of the immunizing infection. The protective efficacy of the live, recombinant anthrax vaccine strains correlated with the anti-PA antibody titers they elicited in vivo and the level of PA they produced in vitro.
Barnes JM (1947). Penicillin and B. anthracis. J Pathol Bacteriol 59: 113-125	Study in mice and rabbits. "Difficulty in controlling infection appears to depend on three factors: (1) production of penicillinase by B. anthracis; (2) persistence of spores in tissues and their germination after the blood-penicillin level has fallen; and (3) poor natural resistance of rabbits and mice to anthrax infection."
Barrow EW, Bourne PC, Barrow WW. (2004). Functional cloning of *Bacillus anthracis* dihydrofolate reductase and confirmation of natural resistance to trimethoprim. Antimicrob Agents Chemother. 48(12):4643-9.	Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma 74078, USA. barrowb@okstate.edu *Bacillus anthracis* is reported to be naturally resistant to trimethoprim (TMP), a drug that inhibits dihydrofolate reductase (DHFR), a key enzyme in the folate pathway. A microdilution broth assay established that the MIC of TMP for B. anthracis Sterne is >2,048 but < or =4,096 microg/ml. A putative DHFR sequence was amplified from B. anthracis Sterne genomic DNA. The PCR product was cloned into the Invitrogen pCRT7/CT-TOPO vector, followed by transformation into Escherichia coli TOP10F' chemically competent cells. Plasmid DNA from a clone showing the correct construct with a thrombin cleavage site attached downstream from the terminus of the cloned PCR product was transformed into E. coli BL21 Star (DE3)pLysS competent cells for expression of the six-histidine-tagged fusion protein and purification on a His-Bind resin column. Functionality of the purified Sterne recombinant DHFR (Sterne rDHFR) was confirmed in an established enzyme assay. The 50% inhibitory concentrations of TMP and methotrexate for the Sterne rDHFR were found to be 77,233 and 12.2 nM, respectively. TMP resistance was observed with E. coli BL21 Star (DE3)pLysS competent cells transformed with the Sterne DHFR gene. Alignment of the amino acid sequence of the Sterne DHFR gene revealed 100% homology with various virulent strains of B. anthracis. These results confirm the natural resistance of B. anthracis to TMP and clarify that the resistance is correlated to a lack of selectivity for the chromosomally encoded gene product. These findings will assist in the development of narrow-spectrum antimicrobial agents for treatment of anthrax.
Barry MR (1971). Changes in animal disease patterns in the Riverina of New South Wales. Aust Vet J 47: 220-225	"Anthrax appears to have declined in recent years. This reduction may not be entirely due to vaccination." Despite lapses in vaccine use, anthrax had not been observed for 40 years on some properties.
Barry WC (1954). The occurrence of anthrax in New Zealand. New Zealand Veterinary Journal 2: 51-52	'It is of interest to note that so far in New Zealand no case of anthrax in sheep has been recorded.'
Bartlett JG (1999). Applying lessons learned from anthrax case history to other scenarios. Emerg Infect Dis 5: 561-563	
Bast DJ, Athamna A, Duncan CL, de Azavedo JC, Low DE, Rahav G, Farrell D, Rubinstein E. (2004). Type II topoisomerase mutations in *Bacillus anthracis* associated with high-level fluoroquinolone resistance. J Antimicrob	Toronto Centre for Antimicrobial Research and Evaluation (ToCARE), Room 1483, Department of Microbiology, Mount Sinai Hospital, 600 University Avenue, Toronto, Canada. dbast@mtsinai.on.ca OBJECTIVES: To identify and characterize the mechanisms of high-level fluoroquinolone resistance in two strains of *Bacillus anthracis* following serial passage in increasing concentrations of fluoroquinolones. METHODS: Fluoroquinolone-resistant isolates of the Sterne and Russian Anthrax Vaccine STi strains were obtained following serial passage in the presence of increasing concentrations of four different fluoroquinolones. The quinolone-resistance-

Chemother. 54(1):90-4.	determining regions of the type II topoisomerase genes from the resistant strains were amplified by PCR and characterized by DNA sequence analysis. The MICs in the presence and absence of reserpine were determined using broth microdilution as a means of detecting active efflux. RESULTS: Single and double amino acid substitutions in the GyrA (Ser-85-Leu; Glu-89-Arg/Gly/Lys) and GrlA (Ser-81-Tyr; Val-96-Ala; Asn-70-Lys) were most common. A single amino acid substitution in GyrB (Asp-430-Asn) was also identified. Efflux only applied to isolates selected for by either levofloxacin or ofloxacin. CONCLUSIONS: Specific amino acid substitutions in the type II topoisomerase enzymes significantly contributed to the development of high-level fluoroquinolone resistance in B. anthracis. However, notable differences between the strains and the drugs tested were identified including the role of efflux and the numbers and types of mutations identified.
Bavykin SG, Lysov YP, Zakhariev V, Kelly JJ, Jackman J, Stahl DA, Cherni A. (2004). Use of 16S rRNA, 23S rRNA, and gyrB gene sequence analysis to determine phylogenetic relationships of Bacillus cereus group microorganisms. J Clin Microbiol. 42(8): 3711-3730	BioChip Technology Center, Argonne National Laboratory, Argonne, IL 60439, USA. sbavykin@anl.gov In order to determine if variations in rRNA sequence could be used for discrimination of the members of the Bacillus cereus group, we analyzed 183 16S rRNA and 74 23S rRNA sequences for all species in the B. cereus group. We also analyzed 30 gyrB sequences for B. cereus group strains with published 16S rRNA sequences. Our findings indicated that the three most common species of the B. cereus group, B. cereus, Bacillus thuringiensis, and Bacillus mycoides, were each heterogeneous in all three gene sequences, while all analyzed strains of Bacillus anthracis were found to be homogeneous. Based on analysis of 16S and 23S rRNA sequence variations, the microorganisms within the B. cereus group were divided into seven subgroups, Anthracis, Cereus A and B, Thuringiensis A and B, and Mycoides A and B, and these seven subgroups were further organized into two distinct clusters. This classification of the B. cereus group conflicts with current taxonomic groupings, which are based on phenotypic traits. The presence of B. cereus strains in six of the seven subgroups and the presence of B. thuringiensis strains in three of the subgroups do not support the proposed unification of B. cereus and B. thuringiensis into one species. Analysis of the available phenotypic data for the strains included in this study revealed phenotypic traits that may be characteristic of several of the subgroups. Finally, our results demonstrated that rRNA and gyrB sequences may be used for discriminating B. anthracis from other microorganisms in the B. cereus group.
Baxter RG (1977). Anthrax in the dairy herd. J S Afr Vet Assoc 48: 293-295	General article reviewing anthrax as a zoonosis and describing transmission, treatment and prevention and environmental disinfection. Refers to report of death of a dairy cow 8 months after vaccination.
Beall FA, Taylor MJ, Thorne CB (1962). Rapid lethal effect in rats of a third component found upon fractionating the toxin of *Bacillus anthracis.* J Bacteriol 83: 1274–80.	[During et al 2001, 10]
Beatty ME, Ashford DA, Griffin PM, Tauxe RV, Sobel J. (2003). Gastrointestinal anthrax: review of the literature. Arch Intern Med. 163(20):2527-31.	Epidemic Intelligence Service, Division of Applied Public Health Training, Epidemiology Program Office, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA. mbeatty@cdc.gov Recent events have drawn attention to cases of inhalational and cutaneous anthrax associated with contaminated mail. Gastrointestinal anthrax, the disease caused by ingestion of *Bacillus anthracis* organisms, has rarely been reported in the United States. This review provides background information on the gastrointestinal form of the disease. We describe the clinical course of gastrointestinal anthrax, outline current therapy, review the microbiology of B anthracis, examine the epidemiology of natural outbreaks, discuss considerations regarding deliberate contamination, and summarize existing literature on the inactivation of spores present in food and water.
Beedham RJ, Turnbull PC, Williamson ED.	Pathobiology, CBD, DERA Porton Down, Salisbury, SP4 0JQ, Wiltshire, UK.

(2001). Passive transfer of protection against *Bacillus anthracis* infection in a murine model. Vaccine. 19(31):4409-16.	Passive transfer of lymphocytes and sera from mice immunised using two different formulations containing recombinant protective antigen (rPA) have been used to further elucidate the mechanism of protection against *Bacillus anthracis* infection. The results demonstrated that an antibody response maybe important in protection against B. anthracis infection, under the conditions tested. The results provide further data for the development of an improved anthrax vaccine.
Beharry Z, Chen HS, Gadhachanda VR, Buynak JD, Palzkill T. (2004). Evaluation of penicillin-based inhibitors of the class A and B beta -lactamases from *Bacillus anthracis*. Biochemical and Biophysical Research Communications 313: 3, 541-545.	*Bacillus anthracis* contains a class A (Bla1) and class B (Bla2) beta -lactamase, which confer resistance to beta -lactam antibiotics when expressed in Escherichia coli. In an effort to find new beta -lactamase inhibitors, several penicillin derivatives have been evaluated including experimental compounds incorporating a 6-mercaptomethyl group or a 6-pyridylmethylidene group, along with clavulanate and tazobactam, as inhibitors against Bla1 and Bla2. The 6-mercaptomethyl-substituted penicillins showed much greater activity against the zinc-containing Bla2 than Bla1. The compound that incorporated a 6-pyridylmethylidene substituent and a catecholic substituent at the 2' position was the most effective inhibitor of Bla1 with Ki=0.057 micro M. Inhibitors containing iron-chelating functional groups have previously been shown to work in combination with antibiotics to inhibit growth of antibiotic-resistant bacteria expressing beta -lactamase. The development of similar compounds, incorporating these types of substituents, may help overcome resistance to currently used antibiotics.
Bell DM, Kozarsky PE, Stephens DS (2002). Clinical issues in the prophylaxis, diagnosis, and treatment of anthrax. Conference summary. Emerg Inf Dis 8: 222-225	Ciprofloxacin, doxycycline, penicillin G – 60 days. Vaccination. PCR of sterile fluids, ELISA – IgG response to PA (high sensitivity 98.6%, 80% specificity); PA competitive inhibition ELISA. Immunohistochemistry.
Bell WJ, Laing PW. (1977). Pulmonary anthrax in cattle Veterinary Record.. 100: 26, 573-574.	Ten days after a case of anthrax in a herd of cows, four cows developed dyspnoea and a drop in milk yield. They had temperatures of 104 deg F; mucous membranes were cyanosed but not injected. The most severe case had oedema of the throat. One cow died before treatment and another after treatment with oxytetracycline and betamethasone; both were confirmed as anthrax cases, though bacilli from the treated cow were vacuolated and capsules had disintegrated. The source of the infection was the dust accompanying the dairy nuts.
Belova EV, Dubilei SA, Kravchenko TB, Kolesnikov AV, Zakharova MIu, Shemiakin IG. (2004). Monoclonal antibodies to B.anthracis protective antigen are capable to neutralize and to enhance the anthrax lethal toxin action in vitro. Mol Gen Mikrobiol Virusol.;(3):21-6.	Anthrax belongs to highly dangerous infections of man and animals. No effective treatment methods for pulmonary types of the disease have been yet developed. The existing anthrax vaccines were designed decades ago and need improvement to fit the large-scale vaccination of population. At the same time, the immunological properties of the anthrax vaccine main component, i.e. of the protective agent, have been poorly studied. We obtained, within the present case study, a panel of mouse monoclonal antibodies to the protective agent and investigated the properties of the highest-affine panel representatives. An unusual phenomenon was detected, which is related with enhancement of the anthrax toxin action on the mouse macrophage-like cell-line in presence of the 1F2 monoclonal antibody. The remaining analyzed antibodies, i.e. 6G8 and 6G7, were found to neutralize effectively the toxin action. The enhancing and neutralizing antibodies were proven to be specific to different domains of the protective antigen and to recognize epitopes in its composition. The antibody-mediated enhancement of the anthrax lethal action is a convincing argument for further development of a new-generation anthrax vaccine. Definition of the linear antigen determinants for neutralizing antibodies in the protective antigens is an important step in the development of the next-generation anthrax vaccine.
Belschner HG (1971). Infectious Diseases 1. Anthrax. *In* Sheep management and	Average number of outbreaks in NSW from1936 to 1957 is 18 per annum 'In the 20 years until 1968 there have been 60 separate outbreaks of anthrax in Victoria with a total of 172 deaths

diseases, 9th edition, Angus and Robertson, Sydney, pp 370-375	recorded. With the exception of 3 sheep, all these deaths were in cattle.' '.. the annual number of deaths from anthrax in NSW is quite low, and when this is considered together with the fact that very few outbreaks occur in Victoria and the disease is either does not occur or is of no significance in other States of Australia, anthrax does not, in this country, assume the importance sometimes attributed to it.'
Belton FC, Strange RE. (1954). Studies on protective antigen produced in vitro from *Bacillus anthracis*: medium and methods of production. Br J Exp Pathol 35: 144-152	
Benavides S, Nahata MC. (2002). Anthrax: safe treatment for children. Ann Pharmacother. 36(2):334-7.	College of Pharmacy, Ohio State University, and Children's Research Institute, Children's Hospital, Columbus, OH, USA. Information regarding the treatment of anthrax infection is scarce in adults and is even more limited in children. Children, however, may be at a greater risk for developing an infection and systemic disease if exposed to anthrax than adults. The Centers for Disease Control and Prevention (CDC) recommends the use of doxycycline or ciprofloxacin for prophylaxis and treatment in children. Doxycycline currently is not indicated for use in children < 8 years old, due to staining of teeth and inhibition of bone growth associated with tetracyclines. Doxycycline, however, may have less adverse effect on teeth than its precursors. Ciprofloxacin has a pediatric indication only when a child is potentially exposed to inhaled anthrax. Ciprofloxacin is contraindicated in pediatric patients because fluoroquinolones were shown to cause cartilage toxicity in immature animals. Although children of various ages have received ciprofloxacin, there are few reports of cartilage toxicity. Because anthrax is a potentially fatal infection, the benefits to using these antibiotics greatly outweigh the risks. Therefore, the use of these antibiotics in children can be recommended, despite the lack of adequate efficacy and safety studies in pediatric patients with anthrax.
Ben-Noun LL. (2003). Figs - the earliest known ancient drug for cutaneous anthrax. Annals of Pharmacotherapy. 37: 2, 297-300.	BACKGROUND: Anthrax is an often fatal bacterial infection, occurring in cutaneous, inhalational, gastrointestinal, and meningeal forms. Evaluation of anthrax treatment from ancient history may help healthcare providers to handle this serious disease more efficiently. OBJECTIVE: To evaluate the biblical descriptions of anthrax, focusing on its therapy in ancient times. STUDY SELECTION: All biblical texts associated with anthrax were examined and passages relating to this disease were studied closely. DATA SYNTHESIS: Biblical passages such as: "Take a cluster of figs. And they took and laid it on the boil [anthrax], and he recovered," and "Let them take a cluster of figs, and lay it upon the boil [anthrax], and he shall recover," convincingly indicate that figs have healing properties in cutaneous anthrax lesions. CONCLUSIONS: On the basis of this study, the National Institutes of Health together with the pharmaceutical industry may consider to work on isolation of compounds from fig plants to develop products against cutaneous and possibly other forms of anthrax once screening tests are completed.
Bentancor LD, Pupillo EA. (1985). Potency of different anthrax vaccines in an outbreak in cattle. Veterinaria Argentina.. 2: 12, 178-179.	
Berry J (2001).Anthrax incident. Animal Health Surveillance (Newsletter of Australia's National Animal Health	Queensland incident, January 2002. Wandoan, Dirranbandi

Information System) 6(4): 3	
Berthold I, Pombo ML, Wagner L, Arciniega JL. (2005). Immunogenicity in mice of anthrax recombinant protective antigen in the presence of aluminum adjuvants. Vaccine.. 23: 16, 1993-1999.	The only US-licensed anthrax vaccine for human use, as well as several experimental vaccines containing solely purified recombinant protective antigen (rPA), are formulated using aluminum hydroxide (Al(OH)3) as an adjuvant. It has been suggested that effective adjuvanticity of aluminum salts for protein antigens depends, at least partially, on the degree of adsorption of the antigen to the adjuvant. On the other hand, the ease of antigen desorption from the adjuvant in a quantitative fashion may facilitate the assessment of vaccine characteristics in the laboratory. In this regard, aluminum phosphate (AlPO4), although deemed a "weaker" adjuvant than Al(OH)3, appears superior to the latter. To investigate the possibility of formulating rPA vaccines with AlPO4, as well as the significance of the adsorption of this antigen to the aluminum salt for adjuvanticity, we studied the effect of AlPO4 and Al(OH)3 on the induction of anti-rPA antibodies in mice. In a first immunization experiment the adjuvanticity of AlPO4 combined with rPA was examined. Antibodies against rPA were measured using an ELISA. Results indicated that AlPO4 is able to significantly increase the antibody response to rPA, irrespective of its degree of adsorption to the adjuvant. Based on these results, in a second experiment mice were immunized twice, with different formulations of rPA containing either AlPO4 or Al(OH)3, and rPA-antibodies were measured using ELISA and an in vitro toxin neutralization assay. Comparable immune responses to rPA were obtained with both aluminum salts. Additionally, results with AlPO4 as adjuvant confirmed that, in this mouse model, binding of the protein to the adjuvant is not essential for adjuvanticity, whereas the amount of adjuvant has an influence on the antibody response induced.
Beveridge WIB (1983). Anthrax. In: Animal Health in Australia, Volume 4. Australian Government Publishing Service, Canberra, 14–19.	Clinical features, pathogenesis, bacteriology, epidemiology, geographic distribution, diagnosis, immunity and vaccination, treatment, prevention and control, anthrax in humans.
Beyer W (2004). Strategies of vaccination against anthrax. Berliner und Munchener Tierarztliche Wochenschrift. 117: 11/12, 508-524.	Apart from live spore vaccines with a certain amount of residual virulence for various animal species, there are two acellular protein vaccines for immunoprophylaxis against anthrax in humans. For ethical reasons there are no experimental data available on the efficacy and duration of the immunity they induce in men. Their efficacy was evaluated in laboratory animals, mainly rabbits and rhesus monkeys. Furthermore, it is well known that these vaccines elicit only partial protection in guinea pigs and almost no protection in mice against a challenge with fully virulent spores of Bacillus (B.) anthracis. Other disadvantages are the high amount of boosters necessary to elicit and to maintain a protective immune response, the variability in the composition of bacterial culture supernatants used for production, and the appearance of clinically relevant side effects. Therefore, there is ongoing work worldwide to improve the existing vaccines by substitution with recombinant antigens and to develop new vaccines on the basis of recombinant bacterial or viral live vectors, DNA-vectors, and by addition of new adjuvants. Special attention is given to supplementing the existing toxoid-vaccines with an anti-bacterial component.
Beyer W, Bartling C, Neubauer H. (2003). The status of methods for the detection of *Bacillus anthracis* in clinical and environmental samples. Tierarztliche Umschau.. 58: 12, 653-662.	It is noted that the detection of B. anthracis in environmental samples is much more difficult than that in clinical samples because of other competing micro-organisms and abiotic factors. This paper reviews the international scientific literature on diagnostic techniques, under the following headings: cultural rearing techniques; light microscopy; electron microscopy; motility tests; phage test; capsule detection; penicillin sensitivity; string-of-pearls test; antigen detection; animal experiments; polymerase chain reaction (PCR). An appendix contains details of methods for cultural detection of B. anthracis, DNA preparations for vegetative cells of the organism, various PCR protocols, semi-selective blood-

	trimethoprim agar, Giemsa rapid staining test for capsule staining, and the Rakette spore staining method.
Beyer W, Glockner P, Otto J, Bohm R. (1995). A nested PCR method for the detection of *Bacillus anthracis* in environmental samples collected from former tannery sites. Microbiological Research.. 150: 2, 179-186.	A nested PCR (polymerase chain reaction) was developed to detect *Bacillus anthracis* spores in natural soil and waste samples which may be heavily contaminated by organic and inorganic compounds as is the case at former tannery sites. Outer and inner pairs of primers were designed from the protective antigen gene of the plasmid pX01 as well as from the genes B and C of the capsule region of the plasmid pX02. The DNA was prepared from an enrichment broth after killing the vegetative cells by H2O2 treatment. The method allows the detection of less than 10 spores per 100 g of the original soil sample. This is between 104-fold and 107-fold more sensitive than the conventional culture diagnosis.
Beyer W, Pocivalsek S, Böhm R. (1999). Polymerase chain reaction-ELISA to detect *Bacillus anthracis* from soil samples: limitations of present published primers. J Appl Microbiol 87: 229-236	
Bhalla DK, Warheit DB. (2004). Biological agents with potential for misuse: a historical perspective and defensive measures. Toxicol Appl Pharmacol. 199(1):71-84.	Department of Fundamental and Applied Sciences, Eugene Applebaum College of Pharm/Health Sci,Wayne State University, Detroit, MI 48202, USA. ad6268@wayne.edu Biological and chemical agents capable of producing serious illness or mortality have been used in biowarfare from ancient times. Use of these agents has progressed from crude forms in early and middle ages, when snakes and infected cadavers were used as weapons in battles, to sophisticated preparations for use during and after the second World War. Cults and terrorist organizations have attempted the use of biological agents with an aim to immobilize populations or cause serious harm. The reasons for interest in these agents by individuals and organizations include relative ease of acquisition, potential for causing mass casualty or panic, modest financing requirement, availability of technology, and relative ease of delivery. The Centers for Disease Control and Prevention has classified Critical Biological Agents into three major categories. This classification was based on several criteria, which include severity of impact on human health, potential for delivery in a weapon, capacity to cause panic and special needs for development, and stockpiling of medication. Agents that could cause the greatest harm following deliberate use were placed in category A. Category B included agents capable of producing serious harm and significant mortality but of lower magnitude than category A agents. Category C included emerging pathogens that could be developed for mass dispersion in future and their potential as a major health threat. A brief description of the category A bioagents is included and the pathophysiology of two particularly prominent agents, namely anthrax and smallpox, is discussed in detail. The potential danger from biological agents and their ever increasing threat to human populations have created a need for developing technologies for their early detection, for developing treatment strategies, and for refinement of procedures to ensure survival of affected individuals so as to attain the ultimate goal of eliminating the threat from intentional use of these agents. International treaties limiting development and proliferation of weapons and continuing development of defense strategies and safe guards against agents of concern are important elements of plans for eliminating this threat. • During WWI, Germany used anthrax (and glanders) to destroy animals used in opposing war efforts • During WWII, in response to fear that German rockets would be loaded with biological agents, Winston Churchill ordered preparations to retaliate by dropping anthrax-contaminated cattle cakes in German countryside to affect beef supply. • Intentional food contaminations – Chilean grapes; salads with S typhimurium; muffins and doughnuts with Shigella dysenteriae
Bhatnagar RN, Mittal KR, Jaiswal TN,	

Padmanaban VD. (1988). Fluctuations in the levels of complement activities, conglutinin, immunoconglutinin and heterohaemagglutinin in the sera of sheep vaccinated with anthrax spore vaccine and challenged with *Bacillus anthracis*. Indian Veterinary Journal.. 65: 11, 959-964.	
Biagini RE, Sammons DL, Smith JP, Page EH, Snawder JE, Striley CA, MacKenzie BA. (2004). Determination of serum IgG antibodies to *Bacillus anthracis* protective antigen in environmental sampling workers using a fluorescent covalent microsphere immunoassay. Occup Environ Med. 61(8):703-8.	Division of Applied Research and Technology, Biomonitoring and Health Assessment Branch, Biological Monitoring Laboratory Section, CDC/NIOSH MS C-26, Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, OH 45226, USA. rbiagini@cdc.gov AIMS: To evaluate potential exposure to Bacillis anthracis (Ba) spores in sampling/decontamination workers in the aftermath of an anthrax terror attack. METHODS: Fifty six serum samples were obtained from workers involved in environmental sampling for Ba spores at the American Media, Inc. (AMI) building in Boca Raton, FL after the anthrax attack there in October 2001. Nineteen sera were drawn from individuals both pre-entry and several weeks after entrance into the building. Nine sera each were drawn from unique individuals at the pre-entry and follow up blood draws. Thirteen donor control sera were also evaluated. Individuals were surveyed for Ba exposure by measurement of serum Ba anti-protective antigen (PA) specific IgG antibodies using a newly developed fluorescent covalent microsphere immunoassay (FCMIA). RESULTS: Four sera gave positive anti-PA IgG results (defined as anti-PA IgG concentrations > or = the mean microg/ml anti-PA IgG from donor control sera (n = 13 plus 2 SD which were also inhibited > or = 85% when the serum was pre-adsorbed with PA). The positive sera were the pre-entry and follow up samples of two workers who had received their last dose of anthrax vaccine in 2000. CONCLUSION: It appears that the sampling/decontamination workers of the present study either had insufficient exposure to Ba spores to cause the production of anti-PA IgG antibodies or they were exposed to anthrax spores without producing antibody. The FCMIA appears to be a fast, sensitive, accurate, and precise method for the measurement of anti-PA IgG antibodies.
Biocorp (2002). Anthrax Vaccine Adsorbed (BioThrax) Package Insert, Lansing, Michigan: BioPort Corporation. January 31, 2002. www.bioportcorp.com	
Blancou JM. (1974). [Study of a mixed vaccine against anthrax and blackleg]. Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux.. 27: 2, 183-187.	A combined vaccine against anthrax and blackleg was prepared with spores of *Bacillus anthracis* spores (attenuated, capsulated) added to whole culture of Clostridium chauvoei killed with propiolactone. After precipitation with 1% potassium alum the vaccine is kept at pH 4. A s/c dose of 2 ml is harmless in cattle and protects against the two diseases. More than 6 million cattle have been vaccinated in Madagascar without any side effects or vaccination failure.
Blumer C, Roche P, Spencer J, Lin Ming; Milton A, Bunn C, Gidding H, Kaldor J, Kirk M, Hall R, Della-Porta T, Leader L, Wright P. (2003). Australia's notifiable diseases status, 2001 - annual report of the National Notifiable Diseases	In 2001, there were 104 187 notifications of communicable diseases in Australia reported to the National Notifiable Diseases Surveillance System (NNDSS). The number of notifications in 2001 was an increase of 16% of those reported in 2000 (89 740) and the largest annual total since the NNDSS commenced in 1991. In 2001, nine new diseases were added to the list of diseases reported to NNDSS and four diseases were removed. The new diseases were cryptosporidiosis, laboratory-confirmed influenza, invasive pneumococcal disease, Japanese encephalitis, Kunjin virus infection, Murray Valley encephalitis virus infection, anthrax, Australian bat lyssavirus, and other lyssaviruses (not

Surveillance System. Communicable Diseases Intelligence.. 27: 1, 1-78.	elsewhere classified). Bloodborne virus infections remained the most frequently notified disease (29 057 reports, 27.9% of total), followed by sexually transmitted infections (27 647, 26.5%), gastrointestinal diseases (26 086, 25%), vaccine preventable diseases (13 030, 12.5%), vectorborne diseases (5294, 5.1%), other bacterial infections (1978, 1.9%), zoonotic infections (1091, 1%) and four cases of quarantinable diseases. In 2001 there were increases in the number of notifications of incident hepatitis C, chlamydial infections, pertussis, Barmah Forest virus infection and ornithosis. There were decreases in the number of notifications of hepatitis A, Haemophilus influenzae type b infections, measles, rubella, Ross River virus infections and brucellosis. This report also summarises data on communicable diseases from other surveillance systems including the Laboratory Virology and Serology Reporting Scheme and sentinel general practitioner schemes. In addition, this report comments on other important developments in communicable disease control in Australia in 2001.
Bohm R (1985). *Bacillus anthracis*. Handbuch der bakteriellen Infektionen bei Tieren, Band V.. VEB Gustav Fischer Verlag, Jena, German Democratic Republic:. 17-89.	A detailed account of the properties of B. anthracis and the epidemiology, clinical features, diagnosis and prophylaxis of anthrax.
Bohm R, Strauch D. (1973). Fluorescent antibody technique for identification of microcultures of *Bacillus anthracis* on membrane filters, as a rapid method of detecting spores. Wiener Tierarztliche Monatsschrift.. 60: Heft 11, 327-332.	In the fluorescent antibody test 4 to 5-hour microcultures of all 20 strains of B. antracis differed significantly in intensity of staining from 21 strains of other aerobic, spore-forming bacilli.
Boor AK, Tresselt HB (1955). Protection of sheep against anthrax by immunisation with antigen prepared in vitro. Am J Vet Res 16: 425-428	One shot vaccination of sheep with PA
Bowen JE, Quinn CP. (1999). The native virulence plasmid combination affects the segregational stability of a theta-replicating shuttle vector in *Bacillus anthracis* var. New Hampshire. Journal of Applied Microbiology.. 87: 2, 270-278.	The segregational stability of a small, theta -replicating, non-mobilizable shuttle plasmid (pAEX-5E) was determined in fully virulent (pX01+/pX02+), partially cured (pX01+/pX02- and pX01-/pX02+) and fully cured (pX01-/pX02-) derivatives of *Bacillus anthracis* var. New Hampshire. Under the growth conditions used (L-broth, 37 degrees C, aerobic, batch culture), pAEX-5E remained segregationally stable in the pX01-/pX02+ and pX01-/pX02- derivatives for in excess of 100 culture generations, but was expelled from the pX01+/pX02+ and pX01+/pX02- derivatives (100% loss occurred after 101 +or- 3.8 and 54 +or- 6.0 culture generations, respectively). In the presence of antibiotic selection pressure to maintain pAEX-5E (5 micro g erythromycin ml-1) no comparable loss of pX01 or pX02 was observed over 100 generations of growth in any of the derivatives of B. anthracis. Under these conditions the pX01+/pX02- derivative had an extended culture doubling time (td +or- S.E. of the mean) of 75.3 +or- 1.4 min compared with 47.3 +or- 1.1, 46.2 +or- 0.86 and 43.2 +or- 1.2 min for the pX01+/pX02+, pX01-/pX02+ and pX01-/pX02- derivatives, respectively. That antibiotic resistance was pAEX-5E-mediated was confirmed using a second antibiotic marker (kanamycin). After 100 generations of growth in the presence of erythromycin, colonies were shown to have retained kanamycin resistance. Southern blot analysis, in conjunction with plasmid rescue to Escherichia coli confirmed that, after 100 culture generations in the presence of antibiotic selection pressure, pAEX-5E had remained structurally stable and had not integrated into the B. anthracis genome.

Bowen JE, Turnbull PCB (1992). The fate of *Bacillus anthracis* in unpasteurised and pasteurised milk. Letters in Applied Microbiology 15:224–227.	Vegetative Ba declined from 5 logs to undetected with 24 hours after to addition to milk (pasteurised or unpasteurised) and kept at 37, 22 or 5-9 degC. Spores of Ba did not decline when stored as above. 'the results provide experimental support for the official view that the public health risks arising from the unlikely event of a cow secreting B. anthracis into the milk are negligible.'
Boyaka PN, Tafaro A, Fischer R, Leppla SH, Fujihashi K, McGhee JR. (2003). Effective mucosal immunity to anthrax: neutralizing antibodies and Th cell responses following nasal immunization with protective antigen. J Immunol. 170(11):5636-43.	Department of Microbiology and Oral Biology, Immunobiology Vaccine Center, University of Alabama, Birmingham, AL 35294, USA. prosper@uab.edu Mucosal, but not parenteral, immunization induces immune responses in both systemic and secretory immune compartments. Thus, despite the reports that Abs to the protective Ag of anthrax (PA) have both anti-toxin and anti-spore activities, a vaccine administered parenterally, such as the aluminum-adsorbed anthrax vaccine, will most likely not induce the needed mucosal immunity to efficiently protect the initial site of infection with inhaled anthrax spores. We therefore took a nasal anthrax vaccine approach to attempt to induce protective immunity both at mucosal surfaces and in the peripheral immune compartment. Mice nasally immunized with recombinant PA (rPA) and cholera toxin (CT) as mucosal adjuvant developed high plasma PA-specific IgG Ab responses. Plasma IgA Abs as well as secretory IgA anti-PA Abs in saliva, nasal washes, and fecal extracts were also induced when a higher dose of rPA was used. The anti-PA IgG subclass responses to nasal rPA plus CT consisted of IgG1 and IgG2b Abs. A more balanced profile of IgG subclasses with IgG1, IgG2a, and IgG2b Abs was seen when rPA was given with a CpG oligodeoxynucleotide as adjuvant, suggesting a role for the adjuvants in the nasal rPA-induced immunity. The PA-specific CD4(+) T cells from mice nasally immunized with rPA and CT as adjuvant secreted low levels of CD4(+) Th1-type cytokines in vitro, but exhibited elevated IL-4, IL-5, IL-6, and IL-10 responses. The functional significance of the anti-PA Ab responses was established in an in vitro macrophage toxicity assay in which both plasma and mucosal secretions neutralized the lethal effects of *Bacillus anthracis* toxin.
Bozhilov B, Nedyalkov S, Gancheva P, Peichev B, Petkov M. (1979). Combined anthrax blackleg vaccine. Veterinarna Sbirka.. 77: 6, 6-9.	[Bulgarian]
Bozhilov B, Petkov M, Ovcharov M. (1978). Standardized vaccine against anthrax in sheep. Veterinarna Sbirka.. 76: 5, 25-26.	[Bulgarian]
Bozhinov B, Petkov M, Gancheva P. (1979). Studies on the sporulation of *Bacillus anthracis*. Veterinarnomeditsinski Nauki.. 16: 9, 29-32.	[Bulgarian] In the sporulation medium of H.U. Kim and M. Goepfert (J. appl. Bact. 37, 265-267; 1974) ten strains of anthrax bacilli showed 99% sporulation within 72 h, but another three strains needed longer than this. The uncapsulated "Ikhtiman" vaccine strain showed 100% sporulation within 24-48 h.
Braack LEO, Retief PF. (1986). Dispersal, density and habitat preference of the blow-flies Chrysomyia albiceps and Chrysomyia marginalis. Onderstepoort J Vet Res;53:13-18.	

Braak LEO, de Vos V (1990). Feeding habits and flight range of blow-flies (Chrysomyia spp.) in relation to anthrax transmission in the Kruger National Park, South Africa. Onderstepoort J Vet Res 57: 141	Blowflies were allow 4 days of feeding on 32P labelled blood or an impala carcase. Dispersal and density of fly faecal and discard droplets was established with a Geiger-Counter. Most droplets occurred between 1 and 3 metres in height on nearby leaves and twigs, the preferred browsing area of kudu who are ofter found most at risk. Trapping also found radiolabelled flies up to 32.5 km from the isotope source.
Brachman PS (1980). Inhalation anthrax. Ann NY Acad Sci;353:83-93.	
Brachman PS, Friedlander AM, Grabenstein JD (2004). Anthrax Vaccine. *In* Vaccines, edited by SA Plotkin & WA Orenstein, 4th edition, Saunders, Philadelphia Chapter 31, pp 887-903	
Brachman PS, Friedlander AM: (1994). Anthrax. In Vaccines edited by SA Plotkin and WA Orenstein, 3rd Edition. Philadelphia, WB Saunders, pp 629-637	
Brachman PS, Gold H, Plotkin SA, Fekety FK, Werrin M, Ingram NR. (1962). Field evaluation of human anthrax vaccine. American Journal of Public Health 52: 632-645	
Brachman PS, Kaufman AF, Dalldorf FG. (1966). Industrial inhalation anthrax. Bacteriology Review 30: 646-659.	
Bradaric N, Punda-Polic V. (1992). Cutaneous anthrax due to penicillin-resistant *Bacillus anthracis* transmitted by an insect bite. Lancet.. 340: 306-307.	This letter reports the case of a 38-year-old woman from Bosnia-Herzegovina who was bitten in the neck by an insect (probably a gadfly). The initial pain and oedema were treated with a histamine antagonist. Two days later, the patient deteriorated and orthostatic hypotension and peripheral vascular collapse developed. On day three, she was transferred to intensive care and the diagnosis of anthrax was made and treatment with penicillin, dopamine, dexamethasone and supportive therapy were initiated. The patient's condition worsened. Culture of the organism from the lesion showed that it was resistant to penicillin, ampicillin and co-trimoxazole but sensitive to tetracycline and aminoglycoside. Antibiotic treatment was changed to tetracycline and defervescence occurred 48 h later and the patient gradually returned to health.
Bradley KA, Mogridge J, Mourez M, Collier RJ, Young JA (2001). Identification of the cellular receptor for anthrax toxin. Nature 414: 225–229.	[Scobie et al 2005, 3] Description of the cloning of the human human PA receptor, ATR (anthrax toxin receptor).

Bradley KA, Young JAT. (2003). Anthrax toxin receptor proteins. Biochemical Pharmacology. . 65: 3, 309-314.	
Bragg TS, Robertson DL. (1989). Nucleotide sequence and analysis of the lethal factor gene (lef) from Bacillus anthracis. Gene 81(1): 45-54	Department of Chemistry, Brigham Young University, Provo, UT 84602. The nucleotide sequence of the Bacillus anthracis lethal factor (LF) gene (lef) has been determined. LF is part of the tripartite protein exotoxin of B. anthracis along with protective antigen (PA) and edema factor (EF). The apparent ATG start codon, which is located immediately upstream from codons which specify the first 16 amino acids (aa) of the mature secreted LF, is preceded by an AAAGGAG sequence, which is its probable ribosome-binding site. This ATG codon begins a continuous 2427-bp open reading frame which encodes the 809-aa LF-precursor protein with an Mr of 93,798. The mature secreted protein (776 aa; Mr 90,237) was preceded by a 33-aa signal peptide which has characteristics in common with leader peptides for other secreted proteins of the Bacillus species. The codon usage of the LF gene reflects its high (70%) A + T content. The N-terminus of LF (first 300 aa) shared extensive homology with the N-terminus of the anthrax EF protein. Since LF and EF each bind PA at the same site, these homologous regions probably represent their common PA-binding domains.
Branch DW, Brozik SM. (2004) Low-level detection of a *Bacillus anthracis* simulant using Love-wave biosensors on 36 degrees YX LiTaO3. Biosensors & Bioelectronics 19: 849–859	Sandia National Laboratories, P.O. Box 5800, MS-0892, Albuquerque, NM 87185-0892, USA We present an acoustic Love-wave biosensor for detection of the *Bacillus anthracis* simulant, Bacillus thuringiensis at or below inhalational infectious levels. The present work is an experimental study of 36◦YX cut LiTaO3 based Love-wave devices for detection of pathogenic spores in aqueous conditions. Given that the detection limit (DI) of Love-wave-based sensors is a strong function of the overlying waveguide, two waveguide materials have been investigated, which are polyimide and polystyrene. To determine the mass sensitivity of Love-wave sensor, bovine serum albumin (BSA) protein was injected into the Love-wave test cell while recording the magnitude and phase shift across each sensor. Polyimide had the lowest mass detection limit with an estimated value of 1.0–2.0 ng/cm2, as compared to polystyrene where DI = 2.0 ng/cm2. Suitable chemistries were used to orient antibodies on the Love-wave sensor using protein G. The thickness of each biofilm was measured using ellipsometry from which the surface concentrations were calculated. The monoclonal antibody BD8 with a high degree of selectivity for anthrax spores was used to capture the non-pathogenic simulant B. thuringiensis B8 spores. Bacillus subtilis spores were used as a negative control to determine whether significant non-specific binding would occur. Spore aliquots were prepared using an optical counting method, which permitted removal of background particles for consistent sample preparation. This work demonstrates that Love-wave biosensors are promising for low-level detection for whole-cell biological pathogens.
Brewer CR, McCullough WG, Mills RC, Roessler WG, Herbst EJ (1946b). Application of nutritional studies for development of practical culture media for Bacillus anthracis. Arch Biochem 10: 77-80	Liquid medium used for large yields of spores (1.5-2*10^9 per ml). Defined medium: tryptic digest of casein, marmite (autolyzed yeast product), distillers solubles, fermentable carbohydrate, potassium phosphate.
Brewer CR, McCullough WG, Mills RC, Roessler WG, Herbst EJ, Howe AF (1946a).Studies on the nutritional requirements of Bacillus anthracis. Arch Biochem 10: 65-75	[Sterne 1959] Metal ions and sporulation. Yields in excess of 10^9 viable spores/ml obtained in chemically defined media. Mn, Ca, Mg, Fe, K, PO4, HCO3 required for or stimulate growth.
Brey RN (2005). Molecular basis for	

improved anthrax vaccines. Advanced Drug Delivery Reviews 57: 1266-1292	
Brook I (2002). The prophylaxis and treatment of anthrax. Int J Antimicrob Agents 20: 320-325	FDA approved: ciprofloxacin, doxycycline, penicillin G Other antibiotics: amoxicillin B anthracis not susceptible to cephalosporins or TMP-sulphonamides
Brook I, Elliott TB, Pryor HI, Sautter TE, Gnade BT, Thakar JH, Knudson GB (2001). In vitro resistance of *Bacillus anthracis* Sterne to doxycycline, macrolides and quinolones. International Journal of Antimicrobial Agents 18: 559–562	
Brookmeyer R, Johnson E, Bollinger R. (2004). Public health vaccination policies for containing an anthrax outbreak. Nature . 432: 7019, 901-904.	Concern about biological weapons has raised questions about the most effective public health policies to contain an anthrax outbreak. We developed a probability model to predict the impact of different anthrax antibiotic and vaccination policies. An anthrax outbreak can be significantly contained by minimizing the delay until initiation of antibiotic prophylaxis. However, even if mass distribution of antibiotics is completed within six days of the initial exposure, then at most about 70% of cases can be prevented. Post-exposure vaccination will not significantly increase that prevention rate if adherence to antibiotic regimens is similar or higher than that attained in the 2001 US outbreak. However, post-exposure vaccination can be useful either in shortening the duration of a prolonged antibiotic regimen, in the event of an antibiotic-resistant strain, or if antibiotic adherence rates are very low. Here we show that a mass pre-exposure vaccination programme for the general population would require very high population coverage rates to significantly increase prevention rates from that achieved with targeted and rapid post-exposure prophylaxis programmes.
Brossier F, Levy M, Mock M. (2002). Anthrax spores make an essential contribution to vaccine efficacy. Infection and Immunity 70: 2, 661-664	Anthrax is caused by *Bacillus anthracis*, a Gram positive spore-forming bacterium. Septicaemia and toxaemia rapidly lead to death in infected mammal hosts. Currently used acellular vaccines against anthrax consist of protective antigen (PA), one of the anthrax toxin components. However, in experimental animals such vaccines are less protective than live attenuated strains. Here we demonstrate that the addition of formaldehyde-inactivated spores (FIS) of B. anthracis to PA elicits total protection against challenge with virulent B. anthracis strains in mice and guineapigs. The toxin-neutralizing activities of sera from mice immunized with PA alone or PA plus FIS were similar, suggesting that the protection conferred by PA plus FIS was not only a consequence of the humoral response to PA. A PA-deficient challenge strain was constructed, and its virulence was due solely to its multiplication. Immunization with FIS alone was sufficient to protect mice partially, and guineapigs totally, against infection with this strain. This suggests that spore antigens contribute to protection. Guinea pigs and mice had very different susceptibilities to infection with the nontoxigenic strain, highlighting the importance of verifying the pertinence of animal models for evaluating anthrax vaccines.
Brossier F, Mock M, Sirard JC. (1999). Antigen delivery by attenuated *Bacillus anthracis*: new prospects in veterinary vaccines. Journal of Applied Microbiology.. 87: 2, 298-302.	Unite Toxines et Pathogenie Bacteriennes, Institut Pasteur, Paris, France. This report summarizes the recent investigations on the use of *Bacillus anthracis* as a live vector for delivery of antigens. Recombinant strains were constructed by engineering the current live Sterne vaccine. This vaccine, used to prevent anthrax in cattle, causes side-effects due to anthrax toxin activities. Bacteria producing a genetically detoxified toxin factor were devoid of lethal effects and were as protective as the Sterne strain against experimental anthrax. Moreover, B. anthracis expressing a foreign antigen controlled by an in vivo inducible promoter were able to generate either antibody or cellular protective responses against heterologous diseases

Brossier F, Weber-Levy M, Mock M, Sirard JC. (2000). Protective antigen-mediated antibody response against a heterologous protein produced in vivo by *Bacillus anthracis*. Infection and Immunity. . 68: 10, 5731-5734.	*Bacillus anthracis* secretes a lethal toxin composed of 2 proteins, the lethal factor (LF) and the protective antigen (PA), which interact within the host or in vitro at the surfaces of eukaryotic cells. Immunization with attenuated B. anthracis strains induces an antibody response against PA and LF. The LF-specific response is potentiated by the binding of LF to PA. In this study, we investigated the capacity of PA to increase the antibody response against a foreign antigen. We constructed a chimaeric gene encoding the PA-binding part of LF (LF254) fused to the C fragment of tetanus toxin (ToxC). The construct was introduced by allelic exchange into the locus encoding LF. Two recombinant B. anthracis strains secreting the hybrid protein LF254-ToxC were generated, one in a PA-producing background and the other in a PA-deficient background. Mice were immunized with spores of the strains, and the humoral response and protection against tetanus toxin were assessed. The B. anthracis strain producing both PA and LF254-ToxC induced significantly higher antibody titres and provided better protection against a lethal challenge with tetanus toxin than did its PA-deficient counterpart. Thus, PA is able to potentiate protective immunity against a heterologous antigen, demonstrating the potential of B. anthracis recombinant strains for use as live vaccine vehicles.
Brossier F, Weber-Levy M, Mock M, Sirard JC. (2000). Role of toxin functional domains in anthrax pathogenesis. Infection and Immunity.. 68: 4, 1781-1786.	The role of the functional domains of anthrax toxins during infection were investigated. Three proteins produced by *Bacillus anthracis*, the protective antigen (PA), the lethal factor (LF), and the oedema factor (OF), combine in pairs to produce the lethal (PA+LF) and oedema (PA+OF) toxins. A genetic strategy was developed to introduce by allelic exchange specific point mutations or in-frame deletions into B. anthracis toxin genes, thereby impairing either LF metalloprotease or OF adenylate cyclase activity or PA functional domains. In vivo effects of toxin mutations were analysed in an experimental infection of mice. A tight correlation was observed between the properties of anthrax toxins delivered in vivo and their in vitro activities. The synergic effects of the lethal and oedema toxins resulted purely from their enzymatic activities, suggesting that in vivo these toxins may act together. The PA-dependent antibody response to LF induced by immunization with live B. anthracis was used to follow the in vivo interaction of LF and PA. It was found that the binding of LF to PA in vivo was necessary and sufficient for a strong antibody response against LF, whereas neither LF activity nor binding of lethal toxin complex to the cell surface was required. Mutant PA proteins were cleaved in mice sera. Thus, the data provide evidence that, during anthrax infection, PA may interact with LF before binding to the cell receptor. Immunoprotection studies indicated that the strain producing detoxified LF and OF, isogenic to the current live vaccine Sterne strain, is a safe candidate for use as a vaccine against anthrax.
Broster MG, Hibbs SE. (1990). Protective efficacy of anthrax vaccines against aerosol challenge. Salisbury Medical Bulletin, special supplement #68, January p 91	
Broughton E. (1992). Anthrax in bison in Wood Buffalo National Park. Can Vet J 33: 134-135.	July 1991: 34 bison (20 males, 9 females, 2 yearlings, 3 calves)
Bruno JG, Hao Yu. (1996). Immunomagnetic-electrochemiluminescent detection of *Bacillus anthracis* spores in soil matrices. Applied and Environmental Microbiology.. 62: 9, 3474-3476.	A rapid method (within about 1.5 hours) for detecting spores of anthrax (*Bacillus anthracis*) in suspensions of soil was accomplished by an immunomagnetic electrochemiluminescence method. Strain-dependent detection limits in buffer were in the range 10superscript 2 to 105 spores. The rank order of sensitivity of the assay for 3 different anthrax strains in buffer was Sterne > Ames > Vollum 1B. Detection was approximately 3 orders of magnitude less sensitive in soil suspensions, and the rank order of sensitivity was altered to Ames > Sterne > Vollum 1B.

Brunsdon JR (1971). Bovine anthrax ante mortem observations. Vet Rec 88: 588	Young dairy cow, normal at morning milking, ill at evening milking and died rapidly. She had been vaccinated with attenuated spore vaccine 8 months previously
Bryskier A. (2002). *Bacillus anthracis* and antibacterial agents. Clinical Microbiology and Infection. 8(8): 467-478	Aventis Pharma SA, Infectious Disease Group, Romainville, France. andre.bryskier@aventis.com Anthrax is one of the oldest threats to humankind, and remains endemic in animals in many parts of the world. Human cases are infrequent, and some result from biological warfare. This review summarizes the current knowledge on the antibacterial activity of available antibiotics. For potential use in the most severe cases of anthrax, antibacterials need to exhibit potent in vitro activity, intracellular bioactivity, and suitable locations in lymph nodes. In animal models, it has been shown that doxycycline and fluoroquinolones are the most active compounds. There is a lack of data for animal models for macrolides and ketolides, some of them exhibiting good in vitro activity. However, systemic anthrax (inhalation or gastrointestinal) is mainly due to anthrax toxin, and therapy directed against intoxication is needed as basic treatment.
Bucchi M. (1997) The public science of Louis Pasteur: the experiment on anthrax vaccine in the popular press of the time. Hist Philos Life Sci. 19(2):181-209.	Department of Sociology, University of Trento, Italy. The paper focuses on Pasteur's public experimentation of the anthrax vaccine (Pouilly-le-Fort, 1881) as portrayed in the English and French popular press of the time. It is argued that this 'popular' level of representation did not merely provide additional publicity for Pasteur's ideas. Rather, the nature and meaning of the experiment itself and of the related controversy on immunisation were substantially negotiated and shaped within the public arena. The multifold consequences of this framing at the public level are explored. In particular, attention is drawn to the relationships that in such process were established with other issues debated at the same time in the arena, namely homeopathy, vivisection and vaccination.
Buchanan TM, Feeley JG, Hayes PS, Brachman PS (1971). Anthrax indirect microagglutination test. J Immunol 107: 1631-1636	[Brachman et al 2004, 123]
Burnett JC, Henchal EA, Schmaljohn AL, Bavari S. (2005). The evolving field of biodefence: therapeutic developments and diagnostics. Nat Rev Drug Discov. 4(4):281-97.	Developmental Therapeutics Program, Target Structure-Based Drug Discovery Group, National Cancer Institute-SAIC, Frederick, Maryland 21702, USA. The threat of bioterrorism and the potential use of biological weapons against both military and civilian populations has become a major concern for governments around the world. For example, in 2001 anthrax-tainted letters resulted in several deaths, caused widespread public panic and exerted a heavy economic toll. If such a small-scale act of bioterrorism could have such a huge impact, then the effects of a large-scale attack would be catastrophic. This review covers recent progress in developing therapeutic countermeasures against, and diagnostics for, such agents.
Bush LM, Abrams BH, Beall A, Johnson CC (2001). Index case of fatal inhalational anthrax due to bioterrorism in the United States. N Engl J Med 345: 1607–10.	[During et al 2001, 1]
Busvine JR. (1980). Insects and hygiene. London: Chapman and Hall,.	
Cairo F, Grande H, Pavan ME, Cervino MJ, Bentancor L. (2002). Bovine anthrax. Departament of Castellanos - Province of Santa Fe. Veterinaria Argentina.. 19:	An anthrax outbreak was reported in January 2002 in cattle in Santa Fe Province, Argentina. This region has not had any previous reports of human or animal anthrax. *Bacillus anthracis* was isolated from a dead animal. The epidemiology and strain characteristics of this outbreak are described. Seventeen deaths were reported in a herd of 1800 non-vaccinated calves (0.94% mortality). The animals presented no symptoms prior to death. After the first deaths, the cattle

184, 285-289.	were vaccinated with the Sterne strain of anthrax vaccine and no further deaths occurred after 4 days. The carcasses were burnt.
Canadian Food Inspection Agency (2003). Anthrax. http://www.inspection.gc.ca/english/anima/heasan/disemala/anthchar/anthcharfse.shmtl	
Carl M, Hawkins R, Coulson N, Lowe J, Robertson DL, Nelson WM, Titball RW, Woody JN. (1992). Detection of spores of *Bacillus anthracis* using the polymerase chain reaction. Journal of Infectious Diseases.. 165: 6, 1145-1148.	The polymerase chain reaction (PCR) was used to identify spores of B. anthracis. By using an assay capable of amplifying a 1247-bp fragment from the gene that encodes the oedema factor of B. anthracis, as few as 10superscript 3 copies of plasmid containing the oedema factor gene and as few as 2 x 104 spores were detected. Subjecting the product of this PCR to a second PCR designed to amplify a 208-bp fragment nested within the 1247-bp product improved detection to a single plasmid copy per PCR and to 2 spores of B. anthracis per PCR.
Carman JA, Hambleton P, Melling J. (1985). *Bacillus anthracis*. *In* Isolation and identification of micro-organisms of medical and veterinary importance, edited by CH Collins and JM Grange, Academic Press, London, UK:. 207-214.	
Carter T (2004). The dissemination of anthrax from imported wool: Kidderminster 1900-14. Occup Environ Med 61: 103-107	Background: A century ago anthrax was a continuing health risk in the town of Kidderminster. The distribution of cases in people and in animals provides an indication of the routes by which spores were disseminated. The response to these cases provides an insight into attitudes to an occupational and environmental risk at the time and can be compared with responses in more recent times. Aims: To assess the distribution of anthrax cases associated with the use of contaminated wool and to review the response to them. Methods: The area studied was Kidderminster, Worcestershire, England, from 1900 to 1914. Data sources were national records of the Factory Inspectorate and local records from the infirmary, Medical Officer of Health and inquest reports, and county agricultural records, supplemented by contemporary and later review articles. Case reports and summary data were analysed, and discussions and actions taken to improve precautions reviewed. Results: There were 36 cases of anthrax, with five deaths, one of which was the sole case of the internal form of the disease. Cases of cutaneous anthrax were most frequently found in those handling raw wool, but they also occurred in workers at later stages of the spinning process and in people with little or no recorded exposure to contaminated wool. Limited precautionary measures were in place at the start of the study period. Some improvements were made, especially in the treatment of infections, but wool with a high risk of anthrax contamination continued to be used and cases continued to arise. Major changes were made to the disposal of waste and to agricultural practice in contaminated areas to curtail outbreaks in farm animals. Conclusions: The introduction of anthrax as a contaminant of imported wool led not only to cases in the highly exposed groups of workers but also to cases in other members of the population and in farm animals. The measures taken during the study period reduced fatalities from cutaneous anthrax but did not eliminate the disease. Public concern about the cases was muted.
Cartwright ME, McChesney AE, Jones RL. (1987). Vaccination-related anthrax in three llamas. Journal of the American Veterinary Medical Association.. 191: 6,	Anthrax vaccine was administered to a herd of 20 llamas following an outbreak of anthrax in cattle on a neighbouring farm. Three 3-month-old llamas became ill (2 of which died) 3 days after inoculation with anthrax spore vaccine, clostridial bacterin toxoid and tetanus toxoid vaccines. Ivermectin was injected at the same time as the vaccines. PM examination of young animals that died revealed abundant *Bacillus anthracis* in lymph nodes and subcutaneous tissue.

715-716.	It is concluded that the Sterne strain vaccine of B. anthracis can cause fatal disease in young llamas. Concurrent administration of ivermectin may have enhanced the infectivity of the vaccine.
Casadevall A (2002). Passive antibody administration (immediate immunity) as a specific defense against biological weapons. Emerg Infect Dis 8: 833-841	[Cherwonogrodzky 2005, 33]
Casadevall A (2005). Antibody-based defense strategies against biological weapons. ASM News, 71: 28-33	[Cherwonogrodzky 2005, 34]
Castillo U, Harper JK, Strobel GA, Sears J, Alesi K, Ford E, Lin J, Hunter M, Maranta M, Ge HY, Yaver D, Jensen JB, Porter H, Robison R, Millar D, Hess WM, Condron M, Teplow D. (203). Kakadumycins, novel antibiotics from Streptomyces sp. NRRL 30566, an endophyte of Grevillea pteridifolia. FEMS Microbiology Letters 224: 2, 183-190.	An endophytic streptomycete (NRRL 30566) is described and partially characterized from a fern-leaved grevillea (Grevillea pteridifolia) tree growing in the Northern Territory of Australia. This endophytic streptomycete produces, in culture, novel antibiotics - the kakadumycins. Methods are outlined for the production and chemical characterization of kakadumycin A and related compounds. This antibiotic is structurally related to a quinoxaline antibiotic, echinomycin. Each contains, by virtue of their amino acid compositions, alanine, serine and an unknown amino acid. Other biological, spectral and chromatographic differences between these two compounds occur and are given. Kakadumycin A has wide spectrum antibiotic activity, especially against Gram-positive bacteria, and it generally displays better bioactivity than echinomycin. For instance, against *Bacillus anthracis* strains, kakadumycin A has minimum inhibitory concentrations of 0.2-0.3 micro g ml-1 in contrast to echinomycin at 1.0-1.2 micro g ml-1. Both echinomycin and kakadumycin A have impressive activity against the malarial parasite Plasmodium falciparum with LD50s in the range of 7-10 ng ml-1. In macromolecular synthesis assays both kakadumycin A and echinomycin have similar effects on the inhibition of RNA synthesis. It appears that the endophytic Streptomyces sp. offer some promise for the discovery of novel antibiotics with pharmacological potential.
Cataldi A, Mock M, Bentancor L. (2000). Characterization of *Bacillus anthracis* strains used for vaccination. Journal of Applied Microbiology.. 88: 4, 648-654.	Three B. anthracis strains, formerly used as anti-anthrax vaccine strains in cattle in Argentina, were characterized. Southern blotting and PCR with pXO1 and pXO2 probes and primers, as well as pathogenicity and protection tests in guinea pigs and mice, were performed. Two of the B. anthracis vaccine strains contained both pXO1 and pXO2 plasmids, as did a fully virulent strain, while the third vaccine strain was a Sterne-type strain (pXO1+, pXO2-). The 3 vaccine strains were, however, less pathogenic than a wild-type virulent strain. It is suggested that the methodology applied here may be used to characterize other B. anthracis strains.
Caudle LC. (1997). The biological warfare threat. *In* Sidel FR, Takafuji ET, Franz DF. (Eds.), Medical Aspects of Chemical and Biological Warfare, pp. 451– 466. Office of the Surgeon General, Department of the Army. Accessible on The Virtual Naval Hospital: http://www.vnh.org/MedAspChemBioWar	[Poisoning of fresh fruits from Chile with cyanide]
Cavallo JD, Ramisse F, Girardet M, Vaissaire J, Mock M, Hernandez E. (2002). Antibiotic susceptibilities of 96 isolates of *Bacillus anthracis* isolated in	Laboratoire de Biologie Medicale, Hopital d'Instruction des Armees Begin, 94163 Saint-Mande, France. Ninety-six isolates of *Bacillus anthracis* recovered in France between 1994 and 2000 were tested for their susceptibilities to 25 different antibiotics. Resistance to penicillin G and amoxicillin was 11.5%. All of the isolates were resistant to cotrimoxazole and susceptible to doxycycline, ciprofloxacin, pefloxacin, levofloxacin, teicoplanin,

France between 1994 and 2000. Antimicrob Agents Chemother. 46(7):2307-9.	vancomycin, clindamycin, imipenem, and rifampin.
CDC (1988). Human cutaneous anthrax-North Carolina, 1987. Morbidity and Mortality Weekly Report. 37: 26, 413-414.	A worker in a North Carolina textile mill developed a sore on his forearm which clinically resembled anthrax although treated at first with an antifungal agent and cephalosporin. Cultures of the sore and blood were negative but he had treatment before specimens were taken. His serum became strongly positive for anthrax antibody. Samples of raw and treated materials and debris were tested and from 8 (14%) of 59 samples anthrax bacilli were grown-5 from West Asian cashmere, 1 from Australian wool and 2 from surface debris in storage area. Anthrax is very rare in the USA: this, in 1987, was the first case since 1984, but the risk remains and exposed workers should be protected by anthrax vaccine.
CDC (2000). Use of anthrax vaccine in the United States: Recommendations fo the Advisory Committee on Immunization Practices (ACIP). MMWR 49(RR-15):1-20.	These recommendations concern the use of aluminum hydroxide adsorbed cell-free anthrax vaccine (Anthrax Vaccine Adsorbed [AVA], BioPort Corporation, Lansing, MI) in the United States for protection against disease caused by *Bacillus anthracis*. In addition, information is included regarding the use of chemoprophylaxis against B. anthracis.
CDC (2001). Update: interim recommendations for antimicrobial prophylaxis for children and breastfeeding mothers and treatment of children with anthrax. MMWR 50: 1014-1016	
CDC (2001b). Update: Investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep. 50(42):909-19.	Erratum in: MMWR Morb Mortal Wkly Rep 2001 Nov 2;50(43):962. Centers for Disease Control and Prevention (CDC). Since October 3, 2001, CDC and state and local public health authorities have been investigating cases of bioterrorism-related anthrax. This report updates previous findings, provides new information on case investigations in two additional areas, presents the susceptibility patterns of *Bacillus anthracis* isolates, and provides interim recommendations for managing potential threats and exposures and for treating anthrax.
CDC (2002). Basic diagnostic testing protocols for Level A laboratories for the presumptive identification of *Bacillus anthracis*. Centers for Disease Control, American Society of Microbiology, Association of Public Health Laboratories. 18 March 2002	Precautions, specimens, QC, Staining, Cultures, Motility Test, interpretation, reporting
CDC (2002). Comprehensive procedures for collecting environmental samples for culturing *Bacillus anthracis*. http://www.bt.cdc.gov	
CDC (2002). Occupational health guidelines for remediation workers at *Bacillus*	Despite the apparently low disease rate from exposure, protection for remediation workers at B. anthracis-contaminated sites is warranted because inhalational anthrax is rapidly progressive and highly fatal, PPE does not guarantee 100%

anthracis-contaminated sites--United States, 2001-2002. MMWR Morb Mortal Wkly Rep. 51(35):786-9.	protection, and the risk for developing disease cannot be characterized adequately. The guidelines described here go beyond HAZWOPER requirements and include recommendations for treating inhalation exposure to B. anthracis spores as a medical emergency, medical follow-up as long as the risk for anthrax persists or a worker is receiving antibiotic prophylaxis, accommodation of a mobile workforce, and assurance that workers understand the need for immediate medical attention should symptoms of anthrax occur. Completion of the 6-dose series of anthrax vaccine followed by annual booster doses will decrease the reliance on antibiotics for the prevention of anthrax. Measures to protect workers must include both medical measures (i.e., vaccination, antibiotic prophylaxis, or a combination of both) and measures to prevent exposure (e.g., PPE and environmental controls).
CDC (2002). Use of anthrax vaccine in response to terrorism: supplemental recommendations of the Advisory Committee on Immunization Practices. MMWR 51: 1024-1026	
Chabot DJ, Scorpio A, Tobery SA, Little SF, Norris SL, Friedlander AM. (2004). Anthrax capsule vaccine protects against experimental infection. Vaccine.. 23: 1, 43-47.	Efficacy of a poly- gamma -D-glutamic acid anthrax capsule vaccine was assessed in a mouse model of infection. Capsule by itself was protective against lethal challenge with a toxin-, capsule+ *Bacillus anthracis* strain. Conjugation of capsule to bovine serum albumin resulted in enhanced IgG anti-capsule antibodies measured by ELISA, but completely abrogated the protection. The protective unconjugated capsule vaccine elicited significantly higher IgM titers and opsonic activity than did the non-protective capsule conjugate. When tested against a fully virulent toxin+, capsule+ B. anthracis strain, neither capsule nor protective antigen alone was protective. However, the combination of the two protected against a lethal challenge. These results suggest that capsule may enhance the protection afforded by protective antigen vaccines against anthrax if opsonizing antibodies are produced. Surprisingly, some protection was also observed when protective antigen was conjugated to itself.
Charlton S, Moir AJG, Baillie L, Moir A. (1999). Characterization of the exosporium of Bacillus cereus. J Appl Microbiol 87: 241-245	
Charney J, Fisher WP, Hegarty CP (1951). Manganese as an essential element for sporulation in the genus Bacillus. J Bacteriol 62: 145-148	[Sterne 1959] Metal ions and sporulation.
Chaudry GJ, Moayeri M, Liu S, Leppla SH. (2002). Quickening the pace of anthrax research: three advances point towards possible therapies. Trends Microbiol 10: 58-62	Identification of cell surface toxin receptor (#6 Bradley et al 2001) Determination of crystal structure of lethal factor protease (#12 Pannifer et al 2001) Susceptibility of inbred mice to lethal toxin associated with mutations in kinesin-like protein Kif1C (#23 Watters et al 2001)
Check E. (2004). BioShield defence programme set to fund anthrax vaccine. Nature. 429(6987):4.	
Chen Y, Succi J, Tenover FC, Koehler TM. (2003). Beta-lactamase genes of the penicillin-susceptible *Bacillus anthracis*	Department of Microbiology and Molecular Genetics, The University of Texas-Houston Health Science Center Medical School, 6431 Fannin Street, Houston, TX 77030, USA. Susceptibility to penicillin and other beta-lactam-containing compounds is a common trait of *Bacillus anthracis*. Beta-

Sterne strain. J Bacteriol. 185(3):823-30.	lactam agents, particularly penicillin, have been used worldwide to treat anthrax in humans. Nonetheless, surveys of clinical and soil-derived strains reveal penicillin G resistance in 2 to 16% of isolates tested. Bacterial resistance to beta-lactam agents is often mediated by production of one or more types of beta-lactamases that hydrolyze the beta-lactam ring, inactivating the antimicrobial agent. Here, we report the presence of two beta-lactamase (bla) genes in the penicillin-susceptible Sterne strain of B. anthracis. We identified bla1 by functional cloning with Escherichia coli. bla1 is a 927-nucleotide (nt) gene predicted to encode a protein with 93.8% identity to the type I beta-lactamase gene of Bacillus cereus. A second gene, bla2, was identified by searching the unfinished B. anthracis chromosome sequence database of The Institute for Genome Research for open reading frames (ORFs) predicted to encode beta-lactamases. We found a partial ORF predicted to encode a protein with significant similarity to the carboxy-terminal end of the type II beta-lactamase of B. cereus. DNA adjacent to the 5' end of the partial ORF was cloned using inverse PCR. bla2 is a 768-nt gene predicted to encode a protein with 92% identity to the B. cereus type II enzyme. The bla1 and bla2 genes confer ampicillin resistance to E. coli and Bacillus subtilis when cloned individually in these species. The MICs of various antimicrobial agents for the E. coli clones indicate that the two beta-lactamase genes confer different susceptibility profiles to E. coli; bla1 is a penicillinase, while bla2 appears to be a cephalosporinase. The beta-galactosidase activities of B. cereus group species harboring bla promoter-lacZ transcriptional fusions indicate that bla1 is poorly transcribed in B. anthracis, B. cereus, and B. thuringiensis. The bla2 gene is strongly expressed in B. cereus and B. thuringiensis and weakly expressed in B. anthracis. Taken together, these data indicate that the bla1 and bla2 genes of the B. anthracis Sterne strain encode functional beta-lactamases of different types, but gene expression is usually not sufficient to confer resistance to beta-lactam agents.
Chen Y, Tenover FC, Koehler TM. (2004). Beta-lactamase gene expression in a penicillin-resistant *Bacillus anthracis* strain. Antimicrob Agents Chemother. 48(12):4873-7.	Department of Microbiology and Molecular Genetics, University of Texas--Houston Medical School, Houston, Texas 77030, USA. Expression of the bla1 and bla2 genes in an archetypal *Bacillus anthracis* strain is insufficient for penicillin resistance. In a penicillin-resistant clinical isolate, both genes are highly transcribed, but bla1 is the major contributor to high-level resistance to ampicillin. Differential expression of the bla genes is dependent upon strain background.
Cherkasskiy BL. (1999). A national register of historic and contemporary anthrax foci. J Appl Microbiol 87:	
Cherwonogrodzky JW (2005). Research strategies for the treatment of biothreats. Current Opinion in Pharmacology 5: 465–472	Department of National Defence, Defence R&D Canada (DRDC Suffield), Chemical and Biological Defence Section, Box 4000 Station Main, Medicine Hat, Alberta T1A 8K6, Canada John.Cherwonogrodzky@drdc-rddc.gc.ca) Whether it is a layperson in the street or a politician in the Senate, there is widespread fear over the consequences of biothreats. In response to these fears, a wide range of treatments has been developed. These include antibiotics (conventional and unconventional uses), nucleic acids (analogues, antisense, ribozymes and DNAzymes), immunomodulators, antibodies, bacteriophage therapy and micro-encapsulation. Furthermore, there are often additional benefits when these therapeutics are used in combination, rather than alone. Although there has been much investment in therapeutics against a terrorist threat for reasons of national security, there are likely to be far greater benefits and applications on domestic and world health.
Cheun HI, Makino SI, Watarai M, Shirahata T, Uchida I, Takeshi K. (2001). A simple and sensitive detection system for *Bacillus anthracis* in meat and tissue. Journal of Applied Microbiology 91: 3,	The objective of this study was to detect and isolate *Bacillus anthracis* from meat and tissue by rapid and simple procedures. *Bacillus anthracis* Pasteur II cells were added to 1 g lymph node and pig meat, which were then cut into small pieces and suspended in phosphate buffered saline (PBS). Aliquots were spread on Bacillus cereus selective agar (BCA) plates to isolate B. anthracis cells, and incubated in trypticase soy broth. The enrichment culture was used for nested polymerase chain reaction (PCR) with B. anthracis specific primers, which were to confirm the presence of B.

421-426.	anthracis chromosomal DNA and the pXO1/pXO2 plasmids. One cell of B. anthracis was detected by nested PCR from 1 g of the samples, and was also isolated on BCA plates according to colony morphology within two days. These results could be useful for detecting animals with latent anthrax, and meat contaminated with B. anthracis, rapidly and simply.
Chin J (editor) (2000). Control of Communicable Diseases Manual, 17th Edition, American Public Health Association, Washington DC.	See Heymann 2004
Choi CS, Kim JS, Chung SI, Yang YT. (1989). Differentiation of attenuated vaccine strains of *Bacillus anthracis*, Pasteur No. 2 Army and Sterne from other bacilli mimicking their biopathological properties. Korean Journal of Veterinary Public Health.. 13: 4, 309.	
Choi CS. (1989). Differential characteristics between virulent isolates and attenuated spore vaccine strains of Pasteur No. 1, No. 2 and Sterne of *Bacillus anthracis* and epidemiology of bovine and human anthrax reported in Korea: 1907-1989. Korean Journal of Veterinary Public Health. 13: 2, 137-147.	
Chopra AP, Boone SA, Liang X, Duesbery NS (2003). Anthrax lethal factor proteolysis and inactivation of MAPK kinase. J Biol Chem 278: 9402–6.	[During et al 2001, 17]
Christopher G, Cieslak T, Eitzen E, (1997). Biological warfare: a historical perspective. JAMA 278, 412– 417.	[Gruinard Island anthrax study]
CIDRAP. (2003). Center for Infectious Disease Research and Policy, 2003 (last updated: September 18, 2003) Anthrax: current, comprehensive information on pathogenesis, microbiology, epidemiology, diagnosis, treatment, and prophylaxis. http://www.cidrap.umn.edu/cidrap/content/bt/anthrax/biofacts/anthraxfactsheet.html	[Postexposure antibiotic prophylaxis]

Cieslak TJ, Christopher GW, Kortepeter MG, Rowe JR, Pavlin JA, Culpepper RC, Eitzen EM Jr. (2000). Immunization against potential biological warfare agents. Clinical Infectious Diseases.. 30: 6, 843-850.	
Cieslak TJ, Eitzen EM (1999). Clinical and epidemiologic principles of anthrax. Emerg Inf Dis 5: 552-555	
Clark R. (1938). Speculation on the incidence of anthrax in bovines. J S Afr Vet Med Assoc 9(1): 5-12	Decrease in immunizing power of anthrax vaccine between 1934 and 1936 preceding introduction of Sterne vaccine.
Clery-Barraud C, Gaubert A, Masson P, Vidal D. (2004). Combined effects of high hydrostatic pressure and temperature for inactivation of *Bacillus anthracis* spores. Applied and Environmental Microbiology. . 70: 1, 635-637.	Spores of *Bacillus anthracis* are known to be extremely resistant to heat treatment, irradiation, desiccation, and disinfectants. To determine inactivation kinetics of spores by high pressure, B. anthracis spores of a Sterne strain-derived mutant deficient in the production of the toxin components (strain RP42) were exposed to pressures ranging from 280 to 500 MPa for 10 min to 6 h, combined with temperatures ranging from 20 to 75 degrees C. The combination of heat and pressure resulted in complete destruction of B. anthracis spores, with a D value (exposure time for 90% inactivation of the spore population) of approximately 4 min after pressurization at 500 MPa and 75 degrees C, compared to 160 min at 500 MPa and 20 degrees C and 348 min at atmospheric pressure (0.1 MPa) and 75 degrees C. The use of high pressure for spore inactivation represents a considerable improvement over other available methods of spore inactivation and could be of interest for antigenic spore preparation.
Cohen S, Mendelson I, Altboum Z (2000). Attenuated nontoxigenic and nonencapsulated recombinant *Bacillus anthracis* spore vaccines protect against anthrax. Infec Immun 68: 4549-4558	134
Coker PR, Smith KL, Fellows PF, Rybachuck G, Kousoulas KG, Hugh-Jones ME. (2003). *Bacillus anthracis* virulence in Guinea pigs vaccinated with anthrax vaccine adsorbed is linked to plasmid quantities and clonality. J Clin Microbiol. 41(3):1212-8.	Department of Pathobiological Sciences. Division of Biotechnology and Molecular Medicine, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA. Coker4@llnl.gov *Bacillus anthracis* is a bacterial pathogen of great importance, both historically and in the present. This study presents data collected from several investigations and indicates that B. anthracis virulence is associated with the clonality and virulence of plasmids pXO1 and pXO2. Guinea pigs vaccinated with Anthrax Vaccine Adsorbed were challenged with 20 B. anthracis isolates representative of worldwide genetic diversity. These same isolates were characterized with respect to plasmid copy number by using a novel method of quantitative PCR developed for rapid and efficient detection of B. anthracis from environmental samples. We found that the copy numbers for both pXO1 and pXO2 differed from those in previously published reports. By combining the data on survival, plasmid copy numbers, and clonality, we developed a model predicting virulence. This model was validated by using a randomly chosen set of 12 additional B. anthracis isolates. Results from this study will be helpful in future efforts to elucidate the basis for variation in the virulence of this important pathogen.
Coker PR, Smith KL, Hugh-Jones ME. (2002). Antimicrobial susceptibilities of	Department of Pathobiological Sciences, Louisiana State University School of Veterinary Medicine, Baton Rouge 70803, USA.

diverse *Bacillus anthracis* isolates. Antimicrob Agents Chemother. 46(12):3843-5.	A test of 25 genetically diverse isolates of *Bacillus anthracis* was conducted to determine their susceptibility to seven clinically relevant antimicrobial agents. Etest strips (AB BIODISK, Solna, Sweden) were used to measure the MICs for the isolates. Using the National Committee for Clinical Laboratory Standards MIC breakpoints for staphylococci, three isolates were found to be resistant to penicillin and five were found to be resistant to cefuroxime. The penicillin-resistant isolates were negative for beta-lactamase production. Continued surveillance of B. anthracis field isolates is recommended to monitor antimicrobial susceptibility.
Cole LA. (2000). Bioterrorism threats: learning from inappropriate responses. J Public Health Manag Pract. 6(4):8-18.	Rutgers University, Newark, New Jersey, USA. Between April 1997 and June 1999, some 200 mailed or telephoned bioterrorism threats were received at a variety of locations. Usually claiming that anthrax had been released, the threats all proved to be hoaxes. In many instances, local emergency responders treated the more than 13,000 potential victims inappropriately, in particular requiring victims to strip and undergo decontamination with bleach solutions. Narratives of several incidents indicated that many victims were distressed and embarrassed by their treatment. Their experiences underscore the need for improved local response actions and the formulation of a uniform response protocol for public health agencies.
Collier RJ. (1999). Mechanism of membrane translocation by anthrax toxin: insertion and pore formation by protective antigen. J Appl Microbiol 87: 283	
Contini A, Bandino E, Ruiu A, Pittau M, Cuccuru C. (1984). Presence of precipitin in guinea pigs inoculated with various *Bacillus anthracis* strains. II. Atti della Societa Italiana delle Scienze Veterinarie.. 38: 728-733.	[Italian] Guinea pigs were inoculated with eleven strains of *Bacillus anthracis* from cattle, sheep, goats and a horse, all of which had died from anthrax. The ID test was used to investigate how soon the antigen was detectable in blood and organs, and how long it persisted there. The test was nearly always negative on samples of liver and muscle, and also negative for some of the strains in blood and spleen. Thus, while positive ID test for B. anthracis infection were reliable, negative ID tests, without confirmation from independent tests, were not.
Costa EF, Fazzio LE, Traveria GE, Sanchez RO, Alvarado Pinedo MF, Mattioli GA, Otero MM, Chialva M, Romero JR. (2004). Causes of mortality and abortion in cattle. Report of 1163 cases from 1986 to 2001 in Buenos Aires, Argentina. Revista de Medicina Veterinaria (Buenos Aires). 85: 1, 16-22.	[Spanish] The cases registered at CEDIVE during 16 years (1986-2001) of consultations involving necropsies and complementary examinations were analyzed in this report. It includes 890 cases of death and 273 of abortion. The conditions were classified by aetiology into ranges of ages. During the first month of life the most important diseases are infectious, with colibacillosis being the most common. Between the 1st and 8th month of age, pneumonia is predominant. In the age range between 8 and 18 months the mucosal disease (BVD-virus), together with anthrax and various parasitic diseases are the most important. Among animals over 18 months, nutritional deficiencies and metabolic diseases became more important, in particular grass tetany. However, among infectious diseases, paratuberculosis, and anthrax are the most frequent. In consultations due to abortion, diagnosis was possible in 35.5% of the cases, in which infectious diseases prevailed and brucellosis was the most important, with campylobacteriosis second.
Cote CK, Rea KM, Norris SL, van Rooijen N, Welkos SL. (2004). The use of a model of in vivo macrophage depletion to study the role of macrophages during infection with Bacillus anthracis spores. Microb Pathog 37(4): 169-175	United States Army Medical Research Institute of Infectious Diseases, Bacteriology Division, 1425 Porter Street, Fort Detrick, Frederick, MD 21702, USA. The pathogenesis of infection by Bacillus anthracis has been the subject of many investigations, but remains incompletely understood. It has been shown that B. anthracis spores germinate in macrophages and perhaps require this intracellular niche to germinate in vivo before outgrowth of the vegetative organism. However, it has also been reported that macrophages are sporicidal in vitro. In our in vivo model, macrophages were depleted from mice by either silica treatment or treatment with liposome-encapsulated dichloromethylene disphosphonate (Cl(2)MDP), and the animals were infected parenterally with virulent ungerminated B. anthracis (Ames strain) spores. The mice in which

	macrophages had been depleted were killed more rapidly than untreated mice. In addition, augmenting peritoneal populations of macrophages with cultured RAW264.7 cells partially protected mice from disease, increasing the survival rate in a dose dependent relationship. Alveolar macrophages were depleted by intranasal instillation of liposome-encapsulated Cl(2)MDP. The animals with normal alveolar macrophage numbers had significantly greater survival rates after inhaling B. anthracis spores than the macrophage-depleted mice. These findings do not preclude the observations that macrophages provide a site permissive for spore germination, however, these data suggest that macrophages do play an important role in limiting and/or clearing a B. anthracis infection.
Cote CK, Rossi CA, Kang AS, Morrow PR, Lee JS, Welkos SL. (2005). The detection of protective antigen (PA) associated with spores of Bacillus anthracis and the effects of anti-PA antibodies on spore germination and macrophage interactions. Microb Pathog 38(5-6): 209-225	United States Army Medical Research Institute of Infectious Disease (USAMRIID), Bacteriology Division, 1425 Porter Street, Fort Detrick, Frederick, MD 21702, USA. The protective antigen (PA) component of the anthrax toxins is an essential virulence factor of Bacillus anthracis and is the major protective immunogen. The kinetics of PA production during growth of B. anthracis, and the roles of anti-PA antibody in host immunity are not clearly defined. Production of PA by the vegetative organisms peaks during the shift from exponential to stationary phase of growth. Recently, PA was also found to be associated with spores. In our study, PA-specific mRNA was detected in spores by RT-PCR within 15-min of exposure to germinant. PA protein was detected by immunomagnetic electrochemiluminescence (ECL) on spores within 1 h of exposure to a germination medium and was rapidly released into the supernatant. PA was not demonstrated on ungerminated spores by RNA analysis, ECL, or spore-based anti-PA ELISA; however, it was detected on ungerminated spores by immunoelectron microscopy (immunoem). In rabbits, PA induces polyclonal antibodies (Abs) that, in addition to their anti-toxin neutralizing activities, exhibit anti-spore activities. In this study, the anti-spore effects of a human monoclonal Ab specific for PA (AVP-hPA mAb, Avanir Pharmaceuticals) were characterized. AVP-hPA mAb retarded germination in vitro, and enhanced the phagocytic and sporicidal activities of macrophages. The activities were comparable to those of the polyclonal rabbit anti-rPA Ab. Assays to detect germination inhibitory activity (GIA) in serum from vaccinated mice and guinea pigs suggested a possible role for anti-PA Abs in protection. Thus, anti-PA Ab-mediated, anti-spore activities may play a role in protection during the early stages of an anthrax infection.
Cottingham K. (2003). A PCR-based anthrax detector. Anal Chem 289	
Coulson NM, Fulop M and Titball RW. (1994). *Bacillus anthracis* protective antigen, expressed in Salmonella typhimurium SL 3261, affords protection against anthrax spore challenge. Vaccine 12:1395-1401	
Coupland R, Henderson J. (1996). Anthrax in northern Alberta. Canadian Veterinary Journal.. 37: 12, 748.	In July 1996, mortalities occurred in a 110 cow-calf pair herd in northern Alberta. A diagnosis of anthrax was confirmed in August following PM examination of a 4-year-old bull within a few hours of death. The herd was quarantined. Burning, burial and liming of carcasses commenced on 10 August. One death was attributed to anthrax a day after vaccination. A bull, 15 cows and 20 calves were included as confirmed or presumptive losses until the time of publishing. Anthrax in bison had occurred sporadically for many years in the nearby Wood Buffalo National Park.
Coupland R, Henderson J. (1996). Anthrax in northern Alberta. Can Vet J 37: 748	July-August 1996: 1 bull, 15 cows and 20 calves died from anthrax. Premise not far from Wood Buffalo National Park.

Coyne SR, Craw PD, Norwood DA, Ulrich MP. (2004). Comparative analysis of the Schleicher and Schuell IsoCode Stix DNA isolation device and the Qiagen QIAamp DNA Mini Kit. Journal of Clinical Microbiology. . 42: 10, 4859-4862.	Efficient, rapid, and reproducible procedures for isolating high-quality DNA before PCR gene amplification are essential for the diagnostic and molecular identification of pathogenic bacteria. This study evaluated the Qiagen QIAamp DNA Mini Kit and the Schleicher and Schuell IsoCode Stix DNA isolation device for isolating nucleic acid. Buffer, serum, and whole-blood samples were spiked with *Bacillus anthracis* Sterne vegetative cells and Yersinia pestis, while water was spiked with B. anthracis Sterne spores. Although minimal variations in limit of detection occurred among matrices, both the IsoCode Stix extraction method and the Qiagen procedure have comparable detection limits.
Cristescu P. (1979). Potency control of anthrax antiserum. Lucrarile Institutului de Cercetari Veterinare si Biopreparate "Pasteur"., publ. 1980. 15: 153-159.	[Romanian] The immunogenicity of five batches of anthrax immune serum, and of serum from repeatedly vaccinated cattle and from unvaccinated control calves was assessed in 30 hamsters and 30 guinea-pigs. In hamsters three batches of immune serum gave 100% protection to challenge with Tenkovski II strain of B. anthracis (520 LD50) and two gave 83% protection; all controls died. In guinea pigs only one serum gave 100% protection, and the others 50-66%; two of the sera at 2 ml gave only 33% protection, but the same sera at 1 ml gave 100% protection; two controls resisted the challenge. Bovine serum gave 33-50% protection in hamsters, but that from the unvaccinated control calves had no immunogenicity. Hamsters are a suitable species for potency tests, but not guinea-pigs.
Crotty S, Aubert RD, Glidewell J, Ahmed R. (2004). Tracking human antigen-specific memory B cells: a sensitive and generalized ELISPOT system. J Immunol Methods. 286(1-2):111-22.	Emory Vaccine Center and Department of Microbiology and Immunology, Emory University School of Medicine, 1510 Clifton Road, Rm G-211, Atlanta, GA 30322, USA. shane@liai.org In the interest of better understanding the role of human memory B cells in protection against disease, we developed an assay to quantitate antigen-specific memory B cells in human blood. This assay utilizes a 6-day polyclonal stimulation of PBMC followed by an antigen-specific ELISPOT for the detection of memory B cells that have differentiated into antibody secreting cells (ASC) in vitro. We have used this assay to demonstrate that the anthrax vaccine (AVA; BioThrax) elicits a substantial population of protective-antigen (PA) specific memory B cells, and these B cells satisfy the canonical surface phenotype of human memory B cells: CD19(+)CD20(+)Ig(+)CD27(+). These anti-PA antigen-specific memory B cells are IgG(+) and represent up to 2% of circulating IgG(+) B cells. Furthermore, these results confirm that vaccine-elicited memory B cells reside in the CD27(+) B cell population. This ELISPOT-based system has been designed in a generalized manner, such that the assay can be rapidly adapted to detect human antigen-specific memory B cells of any given specificity. This method should be useful for quantitatively assessing the potency of vaccines and the longevity of B cell immunological memory to various vaccines or infectious diseases.
Crowther RW, Gambles RM. (1983). Anthrax eradication in Cyprus: an historical survey. Tropical Animal Health and Production.. 15: 2, 103-105.	
Culley NC, Pinson DM, Chakrabarty A, Mayo MS, LeVine SM (2005). Pathophysiological Manifestations in Mice Exposed to Anthrax Lethal Toxin. Infect Immun 73: 7006-7010.	Department of Preventative Medicine and Public Health, University of Kansas Medical Center, Kansas City, Kansas 661603 nculley@kumc.edu Pathophysiological changes associated with anthrax lethal toxin included loss of plasma proteins, decreased platelet count, slower clotting times, fibrin deposits in tissue sections, and gross and histopathological evidence of hemorrhage. These findings suggest that blood vessel leakage and hemorrhage lead to disseminating intravascular coagulation and/or circulatory shock as an underlying pathophysiological mechanism..
Cunha BA. (2002). Anthrax, tularemia, plague, ebola or smallpox as agents of bioterrorism: recognition in the emergency room. Clinical Microbiology and Infection. . 8: 8, 489-503.	Bioterrorism has become a potential diagnostic consideration in infectious diseases. This article reviews the clinical presentation and differential diagnosis of potential bioterrorist agents when first presenting to the hospital in the emergency room setting. The characteristic clinical features of inhalation anthrax, tularaemic pneumonia, plague pneumonia, including laboratory and radiographic finding, are discussed. Ebola virus and smallpox are also discussed as potential bioterrorist-transmitted infections from the clinical and epidemiological standpoint. In addition to the clinical

	features of the infectious diseases mentioned, the article discusses the infectious disease control and epidemiological implications of these agents when employed as bioterrorist agents. The review concludes with suggestions for postexposure prophylaxis and therapy.
Cunningham K, Lacy DB, Mogridge J, Collier RJ. (2002). Mapping the lethal factor and edema factor binding sites on oligomeric anthrax protective antigen PNAS 99: 7049-7053	
Cunningham W (1976). The work of two Scottish medical graduates in the control of woolsorters' disease. Med Hist 20: 169-173	John Henry Bell Frederick William Eurich
Darie P, Ionita C, Petrosanu D, Eustafievici O, Simon M de; Mircescu G. (1979). Simultaneous vaccination of intensively reared lambs against anaerobes, anthrax and foot and mouth disease. Lucrarile Institutului de Cercetari Veterinare si Biopreparate "Pasteur".. 15: 75-86.	[Romanian] A total of 337 lambs were vaccinated with either (a) two doses (2 + 3 ml) 14 days apart or (b) a single dose of 5 ml vaccine against anaerobic toxic bacteria; or 0.2 ml anthrax vaccine plus (a) or (b); or FMD vaccine (3 ml) plus (a) or (b); or tetanus vaccine plus FMD vaccine plus (a) or (b). Efficacy of the vaccine against anaerobic toxic bacteria was determined by serum neutralization of beta and epsilon fractions and Clostridium oedematiens in mice. No interference with the immunogenic effect of the different vaccines was observed.
Darie P, Mircescu G, Simon M de; Petrosanu D, Tetu M, Ionita C. (1979). Simultaneous vaccination of intensively reared lambs against anthrax and foot and mouth disease. Lucrarile Institutului de Cercetari Veterinare si Biopreparate "Pasteur".. 15: 69-74.	[Romanian] After a successful preliminary trial of separate and simultaneous vaccination of a small number of lambs, further trials were carried out with (a) 72 lambs inoculated against anthrax, (b) 54 against FMD, and (c) 107 against both diseases. 45 unvaccinated lambs were controls. Dosage was 0.2 ml anthrax vaccine and 3 ml trivalent AOC vaccine, injected s/c. Artificial challenge revealed 100% immunity against both diseases between 42 and 128 days after vaccination. No adverse effects were observed.
Darlow HM, Belton FC, Henderson DW. (1956). The use of anthrax antigen to immunise man and monkey. Lancet ii: 476-479	
Davies JCA. (1980). Transmission of anthrax. Cent Afr J Med;26:47. Letter.	
Davis RG. (2004). The ABCs of bioterrorism for veterinarians, focusing on Category A agents. Journal of the American Veterinary Medical Association. . 224: 7, 1084-1095.	Definition of bioterrorism: 'The use, or threatened use, of microorganisms or their toxins against humans, animals, or plants by individuals or groups motivated by political, religious, ecological, or other ideological objectives.' On review of published information, there are only 6 confirmed instances worldwide in which terrorist groups used or tried to use biological weapons. Only 2 of these (Rajneeshee attack in 1984, postal mailings in 2001) have resulted in human illness or death. However, other undocumented cases may have occurred Review of anthrax and other CAT A diseases.
de Vos V (1990). The ecology of anthrax in the Kruger National Park, South Africa.	

Proceedings of the International Work shop on Anthrax. Salisbury Medical Bulletin;68(suppl):19-23.	
de Vos V (1994). Anthrax. In: Infectious Diseases of Livestock with Special Reference to Southern Africa (JAW Coetzer, GR Thomson and RC Tustin, eds), Volume 2, Oxford University Press, Oxford, 1262–1289.	1281: (Sterne vaccine) a single inoculation provides effective immunity for about nine months. Effective immunity generally develops within a week of vaccination … … high infection rates may cause disease even in immunized animals.
de Vos V (1998). The isolation of viable and pathogenic *Bacillus anthracis* organisms from 200 + 50 year old bones from the Kruger National Park; a preliminary report. Proceedings of the Agricultural Research Council Onderstepoort OIE International Congress with the World Health Organization co-sponsorship on anthrax, brucellosis, CBPP (contagious bovine pleuro pneumonia) clostridial and mycobacterial diseases, 22–24.	
Dell'Aica I, Dona M, Tonello F, Piris A, Mock M, Montecucco C, Garbisa S. (2004). Potent inhibitors of anthrax lethal factor from green tea. EMBO Reports.. 5: 4, 418-422.	The anthrax lethal factor (LF) has a major role in the development of anthrax. LF is delivered by the protective antigen (PA) inside the cell, where it exerts its metalloprotease activity on the N-terminus of MAPK-kinases. PA+LF are cytotoxic to macrophages in culture and kill the Fischer 344 rat when injected intravenously. We describe here the properties of some polyphenols contained in green tea as powerful inhibitors of LF metalloproteolytic activity, and how the main catechin of green tea, (-)epigallocatechin-3-gallate, prevents the LF-induced death of macrophages and Fischer 344 rats.
Demicheli V, Rivetti D, Decks JJ (1998). The effectiveness and safety of vaccines against human anthrax: a systematic review. Vaccine 16: 880-884	[Brachman et al 2004, 118]
Derbin MI, Garin NS, Tarumov VS, Mikhailov VV, Sadovoy NV, Kuzmich MK, Fedorova NV, Shentsev IV, Muntyanov PV, Kravets ID, Fofanov PE, Ilyukhin VP, Shumilov GP. (1977). Preparation and properties of anthrax protective antigen. IV. Harmlessness, reactogenic properties, and efficacy of concentrated, purified, adsorbed chemical anthrax	

vaccine. Zhurnal Mikrobiologii Epidemiologii i Immunobiologii.. 8, 115-120.	
Derbin MI, Kuzmich MK, Garin NS, Tarumov VS, Mikhailov VV, Fedorova NV, Sodovoy NV, Kravets ID, Shentsev IV. (1977). Preparation and properties of anthrax protective antigen. III. Improved method of obtaining chemical anthrax vaccine under laboratory conditions. Zhurnal Mikrobiologii Epidemiologii i Immunobiologii.. 2, 63-67.	The vaccine was prepared by various methods from 24-30 h cultures of the STI-1 strain of B. anthracis. Protective antigen (PA) was extracted from the bacteria by ethanol precipitation of pH 5.1-5.3, concentrated by four different methods, the most effective being salting-out with ammonium sulphate, or absorption on to ammonium hydroxide gel. The results of sterilizing filtration of the concentrated antigen through a membrane filter, a ceramic candle or an ashestos-cellulose plate were compared. Irrespective of the method of preparation, the vaccines were found to be sterile, innocuous for guinea-pigs, and strongly immunogenic for laboratory animals (mice and rabbits). In gel diffusion tests, the PA was identified as a thermolabile fraction, inactivated by trypsin, unaffected by lysozyme, hyaluronidase or RNA-ase. This thermolabile fraction was resolved into two components on a Sephadex column, the PA being identified with a component of 70 000 molecular weight.
Derbin MI, Mikhailov VV, Tarumov VS, Garin NS, Fedorova NV, Mostov EG, Sadovoy NV, Kuzmich MK, Kravetz,I., Marinin LI, Shentsev IV. (1976). Preparation and properties of anthrax antigen. I. Nutrient medium for production of crude anthrax protective antigen. Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii.. 3, 76-81.	The best culture medium for preparing antigen from *Bacillus anthracis* was one containing 3% hydrochloric acid hydrolysate of fish meal, 4% maize extract, 0.75% glucose, 1.25% sodium bicarbonate and also thiamin; Mg, Fe and Mn sulphates; calcium chloride; potassium phosphate. Crude antigen contained 223 plus or minus 68 immunizing doses (ID50) per ml.
Derflinger ER (1931). Control of anthrax in live stock of the northwest. JAVMA 78: 42-56	
Descotes J.-P, Joubert L. (1978). Current epidemiological recrudescence of anthrax, and the advisability of reenactment of relevant legislation. An outbreak in cattle, with four human cases in Savoy. Revue de Medecine Veterinaire.. 129: 8/9, 1209 . . . 1221.	In 1967/8 there were 14 outbreaks of anthrax in animals and four related cases in man in the Ain Department, caused by feeding cattle infected bone meal imported from Asia. In 1977 six cases of bovine and four of human anthrax occurred in Savoy after reclamation, for growing hay, of a marsh used 40 years previously for dumping anthrax carcasses. The following measures are recommended: strict application of laws relating to the import of animal products and of foodstuffs destined for animal consumption; systematic annual vaccination in areas where drainage and other public works have caused suspect land to be reclaimed; transport of suspect corpses in sealed vehicles; disinfection of infected areas; close co-operation between veterinary and medical authorities when an outbreak occurs.
Devlin LW. (1943). The advent of vaccination of stock against anthrax in Australia: with some early history of anthrax in the Riverina District of New South Wales. Aust Vet J 19: 102-111	
Dewan PK, Fry AM, Laserson K, Tierney BC, Quinn CP, Hayslett JA, (2002). Inhalational anthrax outbreak among postal workers, Washington, D.C., 2001. Emerg Infect Dis 8: 1066-1072.	

Dingle PJ. (1975). An anthrax outbreak. Vet Rec 97: 339-340	Sows treated with penicillin and streptomycin (successful) and cows with penicillin alone (unsuccessful).
Dixon TC, Meselson M, Guillemin J, Hanna PC (1999). Anthrax. New Eng J Med 341: 815-826	
Doganay M, Aydin N. (1991). Antimicrobial susceptibility of *Bacillus anthracis*. Scand J Infect Dis. 23(3):333-5.	Section of Infectious Diseases, Faculty of Medicine, Erciyes University, Kayseri, Turkey. 22 *Bacillus anthracis* isolates were tested for susceptibility to 27 antimicrobial agents by agar dilution. All isolates were sensitive to penicillins and did not produce beta-lactamase. Although all isolates were sensitive to cefazolin, cephalothin, cephradine and cefoperazone 19 isolates were resistant to cefuroxime, 18 to cefotaxime, 18 to ceftizoxime, 9 to ceftriaxone and 21 to ceftazidime. All isolates were also found to be sensitive to other antimicrobials tested. The new antimicrobial agents, ofloxacin and ciprofloxacin showed very good activity with MICs of 0.03-0.06 mg/l.
Dong SL: (1990). Progress in the control and research of anthrax in China. Salisbury Medical Bulletin, special supplement #68, January p 104	
Dorofeev AA, Nesterova YuF. (1976). History of the introduction of "STI" anthrax vaccine (developed by N.N. Ginsburg in 1940). Veterinariya, Moscow. 12, 100-102.	
Drager K, Ackermann O, Barth R, Engelhardt H, Jaeger O, Korner L, Pranter W, Reiche A. (1979). Production of vaccines. Handbuch der bakteriellen Infektionen bei Tieren. Band I. VEB Gustav Fischer Verlag., 69 Jena, German Democratic Republic:. 121-226.	[German]
Drago L, De Vecchi E, Lombardi A, Nicola L, Valli M, Gismondo MR. (2002). Bactericidal activity of levofloxacin, gatifloxacin, penicillin, meropenem and rokitamycin against *Bacillus anthracis* clinical isolates. J Antimicrob Chemother. 50(6):1059-63.	Laboratory of Clinical Microbiology, L. Sacco Teaching Hospital, University of Milan, Via GB Grassi 74, 20157 Milan, Italy. microbio@mailserver.unimi.it This study aimed to evaluate the bactericidal rates of levofloxacin, gatifloxacin, penicillin, meropenem and rokitamycin against seven isolates of *Bacillus anthracis* clinically isolated between 1960 and 1970. After determination of MIC and MBC, time-kill experiments were carried out. Antimicrobial activity was evaluated at concentrations equal to 1 x, 2 x, 4 x and 8 x MIC after 0, 3, 6, 12 and 24 h of incubation with the drugs. Bactericidal activity was defined as a decrease in bacterial count of at least 3 log10. All the isolates were susceptible to all the antibiotics, by considering the antistaphylococcal breakpoints. Levofloxacin was bactericidal at 1 x MIC after 24 h and at 4 x MIC after 12 h, and gatifloxacin was bactericidal at 2 x MIC after 24 h and at 8 x MIC after 12 h. Meropenem, rokitamycin and penicillin also showed bactericidal activity at concentrations of 4 x and 8 x MIC, respectively, but only after 24 h incubation; after the same time, meropenem and rokitamycin showed a more marked killing than penicillin at 2 x MIC.
Dragon DC and Rennie RP (1995). The ecology of anthrax spores: tough but not	

invincible. Canadian Veterinary Journal 36:295–300.	
Dragon DC, Elkin BT, Nishi JS, Ellsworth TR. (1999). A review of anthrax in Canada and implications for research on the disease in northern bison. J Appl Microbiol 87: 208-213	
Dragon DC, Rennie RP and Gates CC (1996). Bison and anthrax in northern Canada. In: Anthrax. Proceedings of an International Workshop, Winchester, 19–21 September 1995 (PCB Turnbull, ed). Salisbury Medical Bulletin, Special Supplement 87:1–2.	
Dragon DC, Rennie RP, Elkin BT (2001). Detection of anthrax spores in endemic regions of northern Canada. J Appl Microbiol 91: 435-441	
Duesbery NS, Vande Woude GF. (1999). Anthrax lethal factor causes proteolytic inactivation of mitogen-activated protein kinase kinase. J Appl Microbiol 87: 289-293	
Duesbery NS, Webb CP, Leppla SH, et al. (1998). Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor. Science 280: 734–7.	[During et al 2001, 11]
Duesbery NS, woude GF vande. (1999). Anthrax toxins. Cel Mol Life Sci 55: 1599-1609	
During RL, Wei Li, Binghua Hao, Koenig JM, Stephens DS, Quinn CP, Southwick FS (2005). Anthrax Lethal Toxin Paralyzes Neutrophil Actin-Based Motility Journal of Infectious Diseases 192: 837–845	*Bacillus anthracis* causes high-level bacteremia, strongly suggesting paralysis of the innate immune system. We have examined the effects of anthrax lethal toxin (LT) on human neutrophil chemotaxis, a process that requires actin filament assembly. Polymorphonuclear neutrophils (PMNs) treated with a sublethal concentration of LT (50 ng/mL) for 2 h demonstrated insignificant apoptosis or necrosis. However, this same concentration slowed human PMN formylmethionylleucylphenylalanine (FMLP)–stimulated chemokinesis by 160%, markedly reduced polar morphology, and rendered PMNs incapable of responding to a chemotactic gradient. These changes were accompanied by a 150% reduction in FMLP-induced actin filament assembly. One hour of exposure to LT failed to impair polarity or actin assembly, and the effects of LT were independent of mitogen-activated protein kinase kinase 1 inhibition. We conclude that 2 h of exposure to LT markedly impairs PMN actin assembly, and reductions in actin filament content are accompanied by a profound paralysis of PMN chemotaxis.

Dzhupina SI, Zhanuzakov NZh, Shushaev BKh. (1982). Prophylaxis of anthrax (among sheep and cattle; antibody titres following immunization with two vaccines). Veterinariya, Moscow. 4, 32-33.	
Ebedes H. (1977). Anthrax epizootics in Etosha National Park. Madoqua.. 10: 2, 99-118.	As a result of vaccination, anthrax is no longer a common disease of domestic animals in South West Africa. In Etosha National Park, however, about 54% of recorded deaths in wild animals are caused by anthrax and 1 635 animals of 10 species are known to have died from anthrax between 1966 and 1974. Contaminated water holes seemed to be the main source of infection. It is suggested that closure of these water holes would be an effective long-term control measure.
Ebright JR, Altantsetseg T, Oyungerel R. (2003). Emerging infectious diseases in Mongolia. Emerg Infect Dis. 9(12):1509-15.	Wayne State University School of Medicine, Detroit, Michigan 48201, USA. jebright@intmed.wayne.edu Since 1990, Mongolia's health system has been in transition. Impressive gains have been accomplished through a national immunization program, which was instituted in 1991. Nevertheless, the country continues to confront four major chronic infections: hepatitis B and C, brucellosis, tuberculosis, and sexually transmitted diseases (STDs). As of 2001, only two cases of HIV infections had been detected in Mongolia, but concern grows that the rate will increase along with the rising rates of STDs and increase in tourism. Other infectious diseases of importance in Mongolia include echinococcosis, plague, tularemia, anthrax, foot-and-mouth, and rabies.
Economides P (2000). Control of zoonoses in Cyprus. Scientific and Technical Review, Office International des Epizooties 19(3):725734.	
Edginton AB (1990). An outbreak of anthrax in pigs: a practioner's account. Veterinary Record 127: 321-324	Outbreak in 500 sow pig unit in Wales in 1989. Vaccine unavailable during outbreak. Parenteral (penicillin) and in-feed (chlortetracycline) antibiotics used. Herd depopulated, facilities fumigated with formalin and slurry decontaminated. Replacement stock vaccinated 4 weeks before entry.
Edginton AB. (1989). Anthrax vaccination. Veterinary Record.. 124: 24, 668.	
Eichhorn A, Lyon BM (1934). A new method of immunization against anthrax. JAVMA 84: 225-232	Description of the Mazzucchi saponin + attenuated No 2 strain vaccine (carbozoo).
Elliott TB, Brook I, Harding RA, Bouhaouala SS, Shoemaker MO, Knudson GB. (2002). Antimicrobial therapy for *Bacillus anthracis*-induced polymicrobial infection in (60)Co gamma-irradiated mice. Antimicrob Agents Chemother. 46(11):3463-71.	Nuclear, Biological, and Chemical Interactions and Countermeasures Research Team, Radiation Medicine Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20889-5603, USA. Elliot@afrri.usuhs.mil Challenge with both nonlethal ionizing radiation and toxigenic *Bacillus anthracis* spores increases the rate of mortality from a mixed bacterial infection. If biological weapons, such as B. anthracis spores, and nuclear weapons were used together, casualties could be more severe than they would be from the use of either weapon alone. We previously discovered that a polymicrobial infection developed in B6D2F(1)/J mice after nonlethal (7-Gy) (60)Co gamma irradiation and intratracheal challenge with B. anthracis Sterne spores 4 days after irradiation. In this present study, we investigated the survival of mice and the response of the polymicrobial infection during the course of antimicrobial therapy with penicillin G procaine, ofloxacin, trovafloxacin, or gatifloxacin. Survival was prolonged, but not ensured,

	when the mice were treated with either broad-spectrum ofloxacin or narrow-spectrum penicillin G for 7 days beginning 6 or 24 h after challenge. Survival was not prolonged when therapy was delayed more than 24 h after challenge. When these two antimicrobial agents were given for 21 days, the survival rate was increased from 0% for the controls to 38 to 63% after therapy. Therapy with trovafloxacin or gatifloxacin reduced the incidence of mixed infection and improved the rate of survival to 95% (trovafloxacin) or 79% (gatifloxacin), whereas the rate of survival for the controls was 5%. We conclude that the mixed infection induced by B. anthracis in irradiated mice complicates effective therapy with a single antimicrobial agent. To limit mortality following nonlethal irradiation and challenge with B. anthracis spores, antimicrobial therapy needs to be initiated within a few hours after challenge and continued for up to 21 days.
Enstone JE, Wale MC, Nguyen-Van-Tam JS, Pearson JC. (2003). Adverse medical events in British service personnel following anthrax vaccination. Vaccine. 21(13-14):1348-54.	Division of Public Health Sciences, University of Nottingham Medical School, Queen's Medical Centre, UK. The safety of the UK anthrax vaccine in British service personnel was evaluated by a retrospective cohort study of randomly selected personnel from five Royal Air Force bases by investigating adverse medical events and consultation rates for a period before and after vaccination. Vaccination acceptance rate varied from 27 to 89% (P=0.0001). In the vaccinated cohort 11.1% (n=368) reported side-effects. The number of consultations in the year prior to vaccination (P=0.04) and RAF base (P=0.0085) were associated with side-effects. Only the RAF base remained a statistically significant factor (P=0.007) after adjusting for other factors. The anthrax vaccine resulted in mild side-effects in 11%, and no serious side-effects were observed. Acceptors of vaccine did not have significantly more medical consultations following vaccination than their unvaccinated counterparts.
Erickson MC, Kornacki JL. (2003). *Bacillus anthracis*: current knowledge in relation to contamination of food. J Food Prot. 66(4): 691-699	Center for Food Safety, Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223, USA. In this article, information related to anthrax and its etiologic agent, *Bacillus anthracis*, in food is reviewed. The major topics discussed include the taxonomic relationship of B. anthracis to other Bacillus species, methods used for the recovery of the organism from surfaces and foods, routes of infection, the pathogenesis of the organism, the microbial ecology of the vegetative cell and spore in foods and the environment, chemical and physical treatments for spore inactivation, and the control of the disease in animals.
Esel D, Doganay M, Sumerkan B (2003). Antimicrobial susceptibilities of 40 isolates of *Bacillus anthracis* isolated in Turkey. International Journal of Antimicrobial Agents 22: 70-72	
Eurich FW (1926). The history of anthrax in the wool industry of Bradford and of its control. Lancet 207: 57-58, 107-110	
Eurich FW, Hewlett RT (1930). *Bacillus anthracis*. *In* A system of bacteriology in relation to medicine, Volume V, HMSO, London. Chapter X, pp 439-478	
Eustafievici O, Balauca N, Cristescu P, Tibrea S. (1982). Potency test of anthrax vaccine in hamsters. Archiva Veterinaria. 16: 171-177	[German] The immune response of young hamsters to anthrax vaccine, prepared with acapsulogenic and oedematogenic 1190 R strain was studied. Nine production batch vaccines and 378 hamsters were used in three experiments. The hamsters were inoculated s/c with decreasing doses: 0.50 ml, 0.25 ml, 0.125 ml, 0.062 ml, and 0.031 ml. The challenge was carried out 15 days after vaccination with a standard suspension of spores in saline solution of Tenkovski II strain, in variable doses of 100-1000 LD50. It was found that the anthrax vaccine given to hamsters

	provided an immunity capable of protecting within the limits of a mean PD50 of 0.062-0.121 ml. The vaccinated hamsters' resistance after challenge was assessed in terms of the vaccine dose and infective dose, a close relationship being found between these two doses.
Ezzell JW Jr, Abshire TG. (1988). Immunological analysis of cell-associated antigens of *Bacillus anthracis*. Infection and Immunity.. 56: 2, 349-356.	Serum from Hartley guinea pigs vaccinated with a veterinary live spore anthrax vaccine was compared with serum from guinea pigs vaccinated with the human anthrax vaccine, which consists of aluminum hydroxide-absorbed culture proteins of *Bacillus anthracis* V770-NP-IR. Serum from animals vaccinated with the spore vaccine recognized two major B. anthracis vegetative cell-associated proteins that were either not recognized or poorly recognized by serum from animals given the human vaccine. These proteins, termed extractable antigens 1 (EA1) and 2 (EA2), have molecular masses of 91 and 62 kilodaltons, respectively. The EA1 protein appeared to be coded by chromosomal DNA, whereas the EA2 protein was only detected in strains with the pX01 toxin plasmid. Both of the extractable antigen proteins were serologically distinct from the components of anthrax oedema toxin and lethal toxin. Following vaccination with the live spore vaccine, the EA1 protein was the predominant antigen recognized, as determined by electrophoretic immunotransblots. Vaccine trials with partially purified EA1 showed that it neither elicits protective antibody against anthrax nor delayed time to death in guinea pigs challenged i.m. with virulent Ames strain spores. Animals vaccinated with sterile gamma-irradiated cell walls developed antibody titres to the N-acetylglucosamine-galactose polysaccharide of B. anthracis but were neither protected nor had a delay in time to death following challenge.
Ezzell JW Jr, Welkos SL. (1999). The capsule of *Bacillus anthracis*, a review. J Appl Microbiol 87: 250	
Farchaus JW, Ribot WJ, Jendrek S, Little SF. (1998). Fermentation, purification, and characterization of protective antigen from a recombinant, avirulent strain of *Bacillus anthracis*. Appl Environ Microbiol. 64(3): 982-991	Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011, USA. dr._joseph_farchaus@ftdetrck-ccmail.army.mil *Bacillus anthracis*, the etiologic agent for anthrax, produces two bipartite, AB-type exotoxins, edema toxin and lethal toxin. The B subunit of both exotoxins is an M(r) 83,000 protein termed protective antigen (PA). The human anthrax vaccine currently licensed for use in the United States consists primarily of this protein adsorbed onto aluminum oxyhydroxide. This report describes the production of PA from a recombinant, asporogenic, nontoxigenic, and nonencapsulated host strain of B. anthracis and the subsequent purification and characterization of the protein product. Fermentation in a high-tryptone, high-yeast-extract medium under nonlimiting aeration produced 20 to 30 mg of secreted PA per liter. Secreted protease activity under these fermentation conditions was low and was inhibited more than 95% by the addition of EDTA. A purity of 88 to 93% was achieved for PA by diafiltration and anion-exchange chromatography, while greater than 95% final purity was achieved with an additional hydrophobic interaction chromatography step. The purity of the PA product was characterized by reversed-phase high-pressure liquid chromatography, sodium dodecyl sulfate (SDS)-capillary electrophoresis, capillary isoelectric focusing, native gel electrophoresis, and SDS-polyacrylamide gel electrophoresis. The biological activity of the PA, when combined with excess lethal factor in the macrophage cell lysis assay, was comparable to previously reported values.
Fasanella A, Losito S, Trotta T, Adone R, Massa S, Ciuchini F, Chiocco D. (2001). Detection of anthrax vaccine virulence factors by polymerase chain reaction. Vaccine. 19(30): 4214-4218	Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Via Manfredonia 20, 71100, Foggia, Italy. izsfoggia@isnet.it In Italy, an attenuated *Bacillus anthracis* strain, named 'Carbosap', is used for immunization against ovine and bovine anthrax. Analysis on 'Carbosap', Sterne vaccine strain F34 and Pasteur vaccine strain SS104, were performed using primers specific for the sequences, encoding the toxic factors, located on plasmids pXO1 and pXO2 and primers specific for the chromosome. The results obtained from polymerase chain reaction (PCR) assay revealed the presence of both plasmids pXO1 and pXO2 in 'Carbosap' strain. This study showed that the 'Carbosap' vaccine strain has a

	different plasmid pattern in comparison to Pasteur vaccine strain SS104 and Sterne vaccine strain F34.
Fedotova YuM. (1974). Role of antitoxin in anthrax immunity. Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii.. 8, 56-59.	Rabbits were immunized by five s/c injections of anthrax protective antigen. Seven days after the last injection, blood samples were taken for antitoxin titration and 50 LD50 of virulent *Bacillus anthracis* was injected i/d. Ten of 22 rabbits survived challenge, and their antitoxin titres ranged from 1:8 to 1:64. Among 12 that died, eight possessed no antitoxin, three had titres of 1:2 and one a titre of 1:4. Antitoxin titre among the survivors 60-80 days after challenge were the same as before challenge in six animals, and had increased in four.
Fee E, Brown TM (2002). Anthrax and the wool trade. Am J Public Health 92: 754-757	
Fellows PF, Linscott MK, Ivins BE, Pitt ML, Rossi CA, Gibbs PH, Friedlander AM. (2001). Efficacy of a human anthrax vaccine in guinea pigs, rabbits, and rhesus macaques against challenge by *Bacillus anthracis* isolates of diverse geographical origin. Vaccine. 19(23-24): 3241-3247	Erratum in: Vaccine 2001 Nov 12;20(3-4):635. Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011, USA. patrica.fellows@amedd.army.mil The efficacy of a licensed human anthrax vaccine (Anthrax Vaccine Adsorbed (AVA)) was tested in guinea pigs, rabbits, and rhesus macaques against spore challenge by *Bacillus anthracis* isolates of diverse geographical origin. Initially, groups of Hartley guinea pigs were vaccinated at 0 and 4 weeks with AVA, then challenged intramuscularly at 10 weeks with spores from 33 isolates of B. anthracis. Survival among the vaccinated groups varied from 6 to 100%, although there were no differences in mean time to death among the groups. There was no correlation between isolate virulence and variable number tandem repeat category or protective antigen genotype identified. New Zealand white rabbits were then vaccinated with AVA at 0 and 4 weeks, and challenged at 10 weeks by aerosol with spores from six of the isolates that were highly virulent in vaccinated guinea pigs. AVA completely protected the rabbits from four of the isolates, and protected 90% of the animals from the other two isolates. Subsequently, two of these six isolates were then used to challenge rhesus macaques, previously vaccinated with AVA at 0 and 4 weeks, and challenged at 10 weeks by aerosol. AVA protected 80 and 100% of the animals from these two isolates. These studies demonstrated that, although AVA confers variable protection against different B. anthracis isolates in guinea pigs, it is highly protective against these same isolates in both rabbits and rhesus macaques.
Fellows PF, Linscott MK, Little SF, Gibbs P, Ivins BE. (2002). Anthrax vaccine efficacy in golden Syrian hamsters. Vaccine. 20(9-10): 1421-1424	Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702-5011, USA. patricia.fellows@amedd.army.mil The efficacy of a licensed human anthrax vaccine (anthrax vaccine adsorbed, AVA) was tested in golden Syrian hamsters against a virulent *Bacillus anthracis* spore challenge. Groups of golden Syrian hamsters were vaccinated at either 0 and 4 weeks or 0, 4 and 8 weeks, then challenged subcutaneously (s.c.) at 10 weeks with spores of various B. anthracis isolates. Although ELISA and toxin neutralization assays demonstrated high titers, none of the AVA-vaccinated hamsters were protected from challenge or demonstrated a significantly extended time to death compared to that of control animals. The results of the study demonstrate that the golden Syrian hamster is not an appropriate model for investigating human anthrax vaccine efficacy.
FEMA (2002). Toolkit for managing the emergency consequences of terrorist incidents. Appendix E: Agriterrorism. Federal Emergency Management Agency, US Government. http://www.fema.gov	Review and history of animal and crop disease threats. Response to the threat of agriterrorism: pp E-20 to E-31 (Includes Agriterrorism Planning Checklist)
Ferrari ME, Hermanson G, Rolland A. (2004). Development of anthrax DNA	Vical Inc, 10390 Pacific Center Court, San Diego, CA 92121, USA. Over 120 years ago, Pasteur and Greenfield developed an in vitro procedure for producing a live-attenuated *Bacillus*

vaccines. Curr Opin Mol Ther. 6(5):506-12.	*anthracis* bacterial culture capable of protecting livestock from anthrax disease. Since then, anthrax has become one of the best characterized bacterial pathogens with regard to mechanism of toxicity and vaccine development. Most developments have used live-attenuated strains, bacterial supernatants or protein subunit approaches. Recently, novel plasmid DNA (pDNA) approaches to a safe and effective anthrax vaccine have been proposed. This review summarizes the history of anthrax, the need for new vaccines and recent developments in pDNA-based vaccines, leading to the initiation of a human phase I clinical trial in a significantly shorter timeframe than in traditional vaccine development.
Flick-Smith HC, Eyles JE, Hebdon R, Waters EL, Beedham RJ, Stagg TJ, Miller J, Alpar HO, Baillie LWJ, Williamson ED. (2002). Mucosal or parenteral administration of microsphere-associated *Bacillus anthracis* protective antigen protects against anthrax infection in mice. Infection and Immunity. 70: 4, 2022-2028.	Existing licensed anthrax vaccines are administered parenterally and require multiple doses to induce protective immunity. This requires trained personnel and is not the optimum route for stimulating a mucosal immune response. Microencapsulation of vaccine antigens offers a number of advantages over traditional vaccine formulations, including stability without refrigeration and the potential for utilizing less invasive routes of administration. Recombinant protective antigen (rPA), the dominant antigen for protection against anthrax infection, was encapsulated in poly-L-lactide 100-kDa microspheres. Alternatively, rPA was loosely attached to the surfaces of microspheres by lyophilization. All of the microspheric formulations were administered to A/J mice with a two-dose schedule by either the intramuscular route, the intranasal route or a combination of these 2 routes, and immunogenicity and protective efficacy were assessed. An intramuscular priming immunization followed by either an intramuscular or intranasal boost gave optimum anti-rPA IgG titres. Despite differences in rPA-specific antibody titres, all immunized mice survived an injected challenge consisting of 103 median lethal doses of *Bacillus anthracis* STI spores. Immunization with microencapsulated and microsphere-associated formulations of rPA also protected against aerosol challenge with 30 median lethal doses of STI spores. These results show that rPA can be encapsulated and surface bound to polymeric microspheres without impairing its immunogenicity and also that mucosal or parenteral administration of microspheric formulations of rPA efficiently protects mice against both injected and aerosol challenges with B. anthracis spores. Microspheric formulations of rPA could represent the next generation of anthrax vaccines, which could require fewer doses because they are more potent, are less reactogenic than currently available human anthrax vaccines, and could be self-administered without injection.
Flick-Smith HC, Waters EL, Walker NJ, Miller J, Stagg AJ, Green M, Williamson ED. (2005). Mouse model characterisation for anthrax vaccine development: comparison of one inbred and one outbred mouse strain. Microb Pathog 38(1): 33-40	Defence Science and Technology Laboratory, Porton Down, Salisbury SP4 0JQ, UK. In order to evaluate the immunogenicity and protective efficacy of anthrax vaccine candidates a suitable small animal model is required. The inbred A/J strain of mouse has been selected as a potential model, and its immune response to immunisation with recombinant protective antigen (rPA) vaccine characterised, by assessment of rPA specific antibody production, and protection against injected challenge, with the unencapsulated STI strain of *Bacillus anthracis*. Studies were conducted to determine the time required post immunisation to develop a protective immune response, to define the minimum protective dose of vaccine required and to assess the long-term immune response to immunisation. From the results of these studies it was possible to establish that the A/J mouse is a consistent and robust small animal model for rPA vaccine testing. A comparison of the immune response to rPA vaccine immunisation in the Turner Outbred (TO) mouse strain was also conducted. Both inbred and outbred mouse strains displayed a predominantly Th2 biased immune response and showed a comparable antibody response to rPA immunisation. An assessment of protection in the TO mouse against aerosol challenge with the fully virulent strain of B. anthracis, Ames, was also made.
Fluck R, Bohm R, Strauch D. (1977). Fluorescent serological studies on cross reactions between spores of *Bacillus anthracis* and spores of other aerobic sporeforming bacteria. Zentralblatt fur	Cross-reactions between spores of B. anthracis and the following aerobic spore-forming bacteria were studied serologically: B. cereus (five strains); B. stubtilis, B. megaterium, B. licheniformis and B. polymyxa (three strains of each); B. thuringiensis, B. laterosporus, B. badius, B. lentus, B. sphaericus, B. panthotenticus and B. psychrosaccharolyticus (one strain each); B. firmus, B. pumilus, B. macerans, B. circulans, B. alvei, B. coagulans and B. globigii (two strains of each). B. cereus, B. pumilus and B. thuringiensis always gave strong cross-reactions, whereas

Veterinarmedizin.. 24B: 6, 497-507.	variable cross-reactions were exhibited with B. megaterium, B polymyxa, B circulans and B coagulans. All the other bacteria gave either weak or no cross-reactions and all the reactions were reversible except in the case of B. polymyxa.
Flynn DM (1968). Anthrax in Victoria. Victorian Veterinary Proceedings 27, 32–33.	
Forino M, Johnson S, Wong TY, Rozanov DV, Savinov AY, Li W (2005). Efficient synthetic inhibitors of anthrax lethal factor. PNAS 102: 9499-9504	
Forshaw D, Higgs ARB, Moir DC, Ellis TM, Links IJ. (1996). Anthrax in cattle in southern Western Australia. Australian Veterinary Journal.. 74: 5, 391-393.	Anthrax was diagnosed in 12 and 4 cattle found dead on 2 neighbouring farms over a period of 7 weeks in February 1994 and in 1 cow on a third farm. All livestock on these farms were vaccinated and each farm was quarantined for 6 weeks after the last death or vaccination. In total 31 cattle died in the outbreak. The mortality rates and the serological results indicated a high exposure to *Bacillus anthracis* on farms 1 and 2. Source of this anthrax outbreak was not identified. Subsequent investigations of 45 suspicious livestock deaths were negative for anthrax and it is concluded that anthrax is not present in the south west of Western Australia except the isolated group of farms in the North Walpole region.
Foster BS, Agahigian DD. (2004). Central serous chorioretinopathy associated with anthrax vaccination. Retina. 24(4):624-5.	New England Retina Consultants, West Springfield, Massachusetts 01089, USA. bsfoster2@yahoo.com
Foster JW, Heiligman F (1949). Mineral deficiencies in complex organic media as limiting factors in the sporulation of aerobic bacilli. J Bacteriol 57: 613-615	[Sterne 1959] Metal ions and sporulation
Fouet A, Mesnage S, Tosi-Couture E, Gounon P, Mock M. (1999). *Bacillus anthracis* surface: capsule and S-layer. J Appl Microbiol 87: 251-255	
Fowler K, McBride BW, Turnbull PCB, Baillie LWJ. (1999). Immune correlates of protection against anthrax. J Appl Microbiol 87: 305	
Fox MD, Boyce JM, Kaufmann AF, Young JB, Whitford HW. (1977) An epizootiologic study of anthrax in Fall Country, Texas. Journal of the American Veterinary Medical Association 170, 327–333.	On individual premises anthrax occurred 89% of deaths occurred within 2 weeks of the 1st death. Of 152 deaths among 2841 cattle on 28 herds, 20.4% occurred 1-16 days before herd vaccination, 19.7% on the day of vaccination, 59.9% occurred 1-20 days after vaccination. Post vaccination deaths occurred at a relatively constant rate for 8 days and then subsided.
Fox MD, Kaufmann AF, Zendel SA, Kolb	

RC, Sengy CG, Cangelosi DA, Fuller CE. (1973) Anthrax in Louisiana, 1971; epizootiological study. Journal of the American Veterinary Medical Association 163, 446–451.	
Frank KJ. (1999). Monitoring temperature-sensitive vaccines and immunologic drugs, including anthrax vaccine. Am J Health Syst Pharm. 56(20): 2052-2055	US Army Medical Materiel Center, Europe, Pirmasens, Germany. frankk@em.pirmasens.amedd.army.mil The experience of the U.S. Army Medical Materiel Center, Europe (USAMMCE), in monitoring temperature-sensitive vaccines and immunologic drugs, including anthrax vaccine, during storage and shipment is discussed. USAMMCE uses an electronic monitoring device to monitor and archive the time-temperature history of shipments of various vaccines, immunoglobulins, and other drugs requiring refrigeration. Using these monitors, USAMMCE can track its carriers' performance, reduce product loss, and validate quality. USAMMCE trains people to pack refrigerated items and to activate and place the monitoring device inside the packing container. Over 1200 temperature-monitor readings from 44 U.S. military logistical depots, hospitals, and clinics located outside the United States are evaluated annually by the USAMMCE pharmacist; each reading represents one shipment or packed box. When deactivated during unpacking, the device flashes green for a successful shipment (all temperature readings within the ideal range) or red for a potentially problematic shipment. From January through October 1998, the device was used in 750 temperature-sensitive shipments; 72% of the devices were returned to USAMMCE in green condition and the remainder in red. Of the red-flashing monitors, 15% were determined to signal that the drugs were received in unacceptable condition. USAMMCE successfully shipped more than 26,000 vials of anthrax vaccine from February through October 1998 within the manufacturer's guidelines for storage temperature. Temperature monitoring is essential for proper storage and transport of vaccines and immunologic drugs.
Frean J, Klugman KP, Arntzen L, Bukofzer S. (2003). Susceptibility of *Bacillus anthracis* to eleven antimicrobial agents including novel fluoroquinolones and a ketolide. J Antimicrob Chemother. 52(2):297-9.	National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa. OBJECTIVES: To determine the susceptibility of southern African strains of *Bacillus anthracis* to new, investigational agents as well as conventional antibiotics. MATERIALS AND METHODS: The MICs of 26 isolates of B. anthracis from South Africa and Zimbabwe, as well as the Sterne vaccine strain and a type culture strain, were determined by agar dilution. RESULTS: The most active antimicrobial agents were the novel ketolide ABT 773, new and conventional fluoroquinolones, and doxycycline; macrolides were intermediately active. The lack of activity of extended-spectrum cephalosporins against B. anthracis was confirmed. CONCLUSIONS: Susceptibility to conventional antibiotics was in keeping with previous studies. Two new fluoroquinolones and a ketolide showed promising in vitro activity that would support their further evaluation in animal models of anthrax.
Freedman ML, Thorpe MEC. (1969). Anthrax: a case report and a short review of anthrax in Australia. Med J Aust 1: 154-157	
Fridman M, Belakhov V, Lee LV, Liang F-S, Wong C-H, Baasov T (2005). Dual effect of synthetic aminoglycosides: antibacterial activity against *Bacillus anthracis* and inhibition of anthrax lethal factor. Angew Chem Int Ed Engl 44: 447-452.	[Cherwonogrodzky 2005, 15]

Friedlander AM (1997). Anthrax. *In* Sidel FR, Takafuji ET, Franz DF. (Eds.), Medical Aspects of Chemical and Biological Warfare, pp. 467-478. Office of the Surgeon General, Department of the Army. Accessible on The Virtual Naval Hospital: http://www.vnh.org/MedAspChemBioWar	
Friedlander AM (1999). Clinical aspects, diagnosis and treatment of anthrax. J Appl Microbiol 87: 303	
Friedlander AM (2000). Anthrax: clinical features, pathogenesis, and potential biological warfare threat. Curr Clin Top Infect Dis 20: 335-349	Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA.
Friedlander AM, Pittman PR, Parker GW. (1999). Anthrax vaccine: evidence for safety and efficacy against inhalational anthrax. JAMA 282(22): 2104-2106	US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011, USA. friedlan@ncifcrf.gov
Fujita O, Inoue S, Tatsumi M, Kamiyama T, Akaishi S, Ootani T, Kawai T, Hirochi T, Sakamoto Y, Tamura K, Watanabe H, Yamada A. (2002). Amplification of irrelevant sequence from Bacillus subtilis using a primer set designed for detection of the pag gene of *Bacillus anthracis*. Japanese Journal of Infectious Diseases. . 55: 3, 99-100.	A "white powder" found disseminated inside a public restroom in Sapporo City, Japan, on 27 March 2002, was examined. Bacteria were isolated suspected to be *Bacillus anthracis*. To confirm, a real-time polymerase chain reaction (PCR) using a LightCycler-B. anthracis Detection Kit was performed. No anthrax-specific signals were detected when the sample was tested for the presence of pag and cag genes. The amplicon was sequenced after cloning it into the pCR2.1 plasmid. The nucleotide sequence showed substantial homology with B. subtilis ATP-dependent deoxyribonuclease, but no homology with any known B. anthracis genes was determined. An increase in the annealing temperature had no effect on the production of these bands when the sample was subjected to PCR using the same pag primer set. The results of biochemical tests indicated that the bacteria isolated belonged to the B. subtilis group. These findings indicate that the PCR protocol used in this study (recommended by WHO) is not sufficiently specific since there remains the possibility that a bacterium exists whose genome contains sequence(s) amplifiable by the primers. Moreover, the size of the amplifiable fragments is indistinguishable from those amplified from B. anthracis.
Galeano B, Korff E, Nicholson WL. (2003). Inactivation of vegetative cells, but not spores, of *Bacillus anthracis*, B. cereus, and B. subtilis on stainless steel surfaces coated with an antimicrobial silver- and zinc-containing zeolite formulation. Applied and Environmental Microbiology.. 69: 7, 4329-4331.	Stainless steel surfaces coated with paints containing a silver- and zinc-containing zeolite (AgION antimicrobial) were assayed in comparison to uncoated stainless steel for antimicrobial activity against vegetative cells and spores of three Bacillus species, namely, B. anthracis Sterne, B. cereus T, and B. subtilis 168. Under the test conditions (25 degrees C and 80% relative humidity), the zeolite coating produced approximately 3 log10 inactivation of vegetative cells within a 5- to 24-h period, but viability of spores of the three species was not significantly affected.
Galloway D, Liner A, Legutki J, Mateczun A, Barnewall R, Estep J. (2004). Genetic immunization against anthrax. (Special	The objective of this study was to determine whether a DNA prime-protein boost immunization against the *Bacillus anthracis* protective antigen (PA) and lethal factor (LF) antigens could induce a protective immune response against significant aerosol challenge in the rabbit model. Rabbits were vaccinated with different regimens of DNA vaccines and

issue: DNA vaccines: research frontiers and clinical applications.) Vaccine 22: 13/14, 1604-1608.	aerosol challenged with B. anthracis spores, Ames strain, with an average dose of 50 LD50s with a range from 18 to 169 LD50s. Of the five vaccinated rabbits that survived, two were immunized intramuscularly (i.m.) with DNA followed with a protein boost and three were immunized subcutaneous (s.q.) with recombinant protein. A major factor predicting survival was the ability of the animal to mount a lasting antibody response to PA. Rabbit sera were collected prior to and following aerosol challenge and titrated for PA antibodies by indirect ELISA. The results of this study indicate that DNA-based immunization against PA and LF induces significant protective immunity against aerosol challenge in the rabbit model and compares favorably with protein-based immunization.
Galvin J (1997). An unusual outbreak of anthrax in Victoria. Animal Health Surveillance (Newsletter of Australia's National Animal Health Information System) 2(1): 4-6	Tatura outbreak
Ganiere JP. (2001). Anthrax: human and animal disease. Point Veterinaire.. 32: 221, 48-51.	
Gardner AD (1945). Microscopical effect of penicillin on spores and vegetative cells of bacilli. Lancet 245(6352): 658-659	Penicllin attacks B anthracis spores at the early stage of germination. In a non-nutient medium spores are little affected by penicillin.
Garmory HS, Titball RW, Griffin KF, Hahn U, Bohm R, Beyer W. (2003). Salmonella enterica serovar typhimurium expressing a chromosomally integrated copy of the *Bacillus anthracis* protective antigen gene protects mice against an anthrax spore challenge. Infect Immun. 71(7): 3831-3836	Defence Science and Technology Laboratory, Salisbury SP4 0JQ, United Kingdom. hsgarmony@dstl.gov.uk Protective immunity against infection with *Bacillus anthracis* is almost entirely based on a response to the protective antigen (PA), the binding moiety for the two other toxin components. We cloned the PA gene into an auxotrophic mutant of Salmonella enterica serovar Typhimurium as a fusion with the signal sequence of the hemolysin (Hly) A gene of Escherichia coli to allow the export of PA via the Hly export system. To stabilize the export cassette, it was also integrated into the chromosome of the live Salmonella carrier. When S. enterica serovar Typhimurium with the chromosomally integrated PA gene was given intravenously to A/J mice, they developed high levels of antibody to PA. These mice were protected against intraperitoneal challenge with 100 or 1,000 50% lethal doses of B. anthracis strain STI. This work contributes to the development of a Salmonella-based orally delivered anthrax vaccine.
Gaur R, Gupta PK, Banerjea AC, Singh Y. (2002). Effect of nasal immunization with protective antigen of *Bacillus anthracis* on protective immune response against anthrax toxin. Vaccine 20(21-22): 2836-2839	Centre for Biochemical Technology Mall Road, Near Jubilee Hall, Delhi-110 007, India. Anthrax toxin consists of three proteins: protective antigen (PA), lethal factor (LF) and edema factor (EF). PA in combination with LF (lethal toxin) is lethal to mammalian cells and is the major component of human anthrax vaccine. Immunization with PA elicits the production of neutralizing antibodies that form a major component of the protective immunity against anthrax. Recent reports have shown that neutralizing antibody titres can serve as a reliable surrogate marker for protection against anthrax. In the present study, the use of non-invasive routes such as bare skin and nose for immunization with PA on its protective immune response was investigated. Mice were inoculated intranasally (i.n.), subcutaneously (s.c.) or through the skin on days 0, 15 and 28 with purified PA. Intranasal and subcutaneous immunization with PA resulted in high IgG ELISA titers. The predominant subclass in each group was IgG1. High titres of IgA were observed only in i.n. immunized mice. In a cytotoxicity assay these sera protected J774A.1 cells from lethal toxin challenge. The results suggest that non-invasive nasal immunization may be useful in improving vaccination strategies against anthrax.
Gavrilov VA. (1987). Immunogenetic aspects of the development of anthrax	

vaccines, a review. Veterinariya, Moscow.. 10, 27-29.	
General Accounting Office (2002). GAO-02-987, Childhood Vaccines: Ensuring an Adequate Supply Poses Continuing Challenges. September 2002.	www.gao.gov/new.items/d02987.pdf
General Accounting Office. (2005). Anthrax detection. GAO-05-251. Washington DC, March 31 2005.	Coordinated interagency approach recommended. Validation needs to be defined and validation activities put in place.
George S, Mathai D, Balraj V, Lalitha MK, John TJ. (1994). An outbreak of anthrax meningoencephalitis. Trans R Soc Trop Med Hyg. 88(2): 206-207	Department of Medicine, Christian Medical College and Hospital, Vellore, Tamil Nadu, India. We report a common-source outbreak of anthrax meningoencephalitis in Chittoor district in Andhra Pradesh, southern India, in October 1990. The source of infection was the carcass of a sheep. Of 5 persons who skinned and cut up its meat for human consumption, 4 developed anthrax meningoencephalitis and one a malignant pustule. Another person who wrapped the meat in a cloth and carried it home on his head developed a malignant pustule on his forehead and also meningoencephalitis. All subjects with anthrax meningoencephalitis died, but the one with only a malignant pustule recovered. A large number of people who cooked or ate the cooked meat of the dead sheep remained well. The medical, public health and veterinary authorities were alerted and sheep, goats and cattle in the locality were immunized with anthrax vaccine. Although rules against consumption of meat of dead animals exist, their violation shows a lack of public awareness. Health education should be undertaken to correct this situation.
Gibbons DF, Hussaini SN. (1974). Isolation of *Bacillus anthracis* from an aborted bovine foetus. Nature.. 252: 5484, 612.	B. anthracis was isolated from a foetus aborted 13 days after the cow had been vaccinated with a spore vaccine to control an outbreak of anthrax in the herd. Two vaginal swabs and a faecal sample taken from the cow 3 days after abortion did not yield B. anthracis. These findings suggest that the foetus becomes infected during the bacteraemia/pyrexia phase of the dam, and that the bacilli continue to multiply in and cause the death of the foetus despite the recovery of the cow. The products of abortion from cows surviving an outbreak of anthrax must be regarded as potentially infected.
Gill IJ (1982). Antibiotic therapy in the control of an outbreak of anthrax in dairy cows. Australian Veterinary Journal.. 58: 5, 214-215.	An outbreak of anthrax in a dairy herd in Victoria is reported. Morbidity was 20%. When the outbreak was confirmed all cattle on the farm were vaccinated, but cows continued to die from the infection. Later all cows were treated with 2500 mg procaine penicillin and 2500 mg benethamine penicillin, after which only one more cow died. Although treatment of the whole herd with antibiotics appeared to curb the outbreak, it is only recommended for herds with very valuable animals to justify the cost of having to discard the milk.
Gillespie JH, Timoney JF. (1981). The genus bacillus. In: Hagan and Bruner's infectious diseases of domestic animals. Ithaca: Cornell University Press,;190-197.	
Gilson RT, Schissel DJ. (2004). Recurrent, localized urticaria and erythema multiforme: a review and management of	Wright-Patterson Medical Center, Wright-Patterson Air Force Base, Ohio 45433, USA. robert.gilson@wpafb.af.mil The October 2001 domestic anthrax attacks affected 22 people, resulting in 5 fatalities. The added global terrorist threats have created an increasing need for homeland protection, as well as protection of our widely deployed forces

cutaneous anthrax vaccine-related events. Cutis. 73(5):319-25.	battling terrorism. It is now relevant for physicians to be familiar with both clinical anthrax and adverse vaccine-related events associated with the resumption of the anthrax vaccine program. Dermatologists played a lead role in the initial response to the anthrax attack. We must be the lead providers most familiar with the cutaneous reactions that may be seen with the preventive vaccination. This article reviews the latest recommended evaluation and management of anthrax vaccine adverse events.
Gladstone GP (1946). Immunity to anthrax: protective antigen in cell free culture filtrates. Brit J Exper Pathol 17: 394-410	"Experiments recorded in this paper show that a highly effective immunizing antigen is produced extracellularly in cultures of B anthracis under certain conditions." Studies in sheep: three doses of 10ml sheep PCF (plasma culture filtrate) s/c protected all sheep against 125 ALD spores injected one week later. Three controls died in 3-4 days.
Gladstone GP (1948). Immunity to anthrax.. Production of the cell-free protective antigen in cellophane sacs. Br J Exp Pathol 24: 379-389	New method of culturing Ba in sacs suspended in sterile broth that was continuously flowing over the sac. Aeration was provided continuously. Yield of antigen was about 25X greater than that produced on "medical flats"
Gladstone GP, Fildes P (1940). A simple culture medium for general use without meat extract or peptone. Brit J Exp Pathol 21(4): 161-173	[Sterne 1946] Description of the medium (CCY – casein-casein-yeast – which contains no meat extract or peptone) used to produce the Sterne vaccine.
Gladus MA, Nikolaenko IuP, Lazareva ES, Fatkhinurova TI, Mashilova GM. (1978). Standardization of the seeding material in producing live anthrax vaccine. Zh Mikrobiol Epidemiol Immunobiol (9): 42-46	Technology of obtaining dry concentrated seeding material of the anthrax bacillus STI-1 vaccine strain was worked out. The use of dry seeding material for making dry anthrax vaccine rendered the preparations obtained more standard, reduced the time required for their production, led to increase of AKM-SH productivity, and to greater profitability of the vaccine production. The vaccine preparations obtained with the use of dry seeding material did not differ from control by immunogenicity.
Glassman HN (1958) World incidence of anthrax in man. Public Health Reports 73: 22	
Gold H (1935). Studies on anthrax. Clinical report of ten human cases. J Lab Clin Med 21: 134-153	
Gold H (1955). Anthrax: a report of one hundred seventeen cases. Arch Intern Med 96: 387-396	88
Goodman L. (2004). Taking the sting out of the anthrax vaccine. J Clin Invest 114(7): 868-869	
Grabenstein JD. (2003). Anthrax vaccine: a review. Immunol Allergy Clin North Am. 23(4):713-30.	US Army Medical Command, 5111 Leesburg Pike, Falls Church, VA 22041, USA. john.Grabenstein@amedd.army.mil Anthrax can be a deadly disease if treatment does not begin early in the course of infection. An effective vaccine has been available in the United States since 1970, although it was not used widely until 1998. A comprehensive, peer-

	reviewed evaluation by the National Academy of Sciences affirmed the findings of multiple previous independent panels that found that the US-licensed anthrax vaccine is safe and effective.
Gracey JF, Collins DS, Huey (1999). Meat Hygiene, 10th Edition. WB Saunders, London, 507-509	
Grasoiu G, Bojoi P, Faur G, Mircescu S. (1973). [Immunization against anthrax with precipitated antigen]. Lucrarile Institutului de Cercetari Veterinare si Biopreparate 'Pasteur'.. 9: 193-206.	[Romanian] The antigen was precipitated by alum, from cultures of the oedematogenous, acapsulogenic strains 1190 R and F2 of *Bacillus anthracis*, or of the capsulogenic strain A95. In biological tests, three 1 ml doses of the precipitated antigen, with an adjuvant (Freund's or Al(OH)3) protected 50-84% of guinea pigs against challenge, while two 5 ml doses gave complete protection to sheep.
Greenberg B. (1971). Flies and animal disease. Vol. 2. Princeton: Princeton University Press,;300-305.	
Greenfield RA, Bronze MS. (2003). Prevention and treatment of bacterial diseases caused by bacterial bioterrorism threat agents. Drug Discovery Today. 8: 19, 881-888.	There is general consensus that the bacterial agents or products most likely to be used as weapons of mass destruction are *Bacillus anthracis*, Yersinia pestis, Francisella tularensis and the neurotoxin of Clostridium botulinum. Modern supportive and antimicrobial therapy for inhalational anthrax is associated with a 45% mortality rate, reinforcing the need for better adjunctive therapy and prevention strategies. Pneumonic plague is highly contagious, difficult to recognize and is frequently fatal. Therefore, the development of vaccines against this agent is crucial. Although tularemia is associated with low mortality, the highly infectious nature of aerosolized F. tularensis poses a substantive threat that is best met by vaccine development. Safer antitoxins and a vaccine are required to meet the threat of the use of botulinum toxin as a weapon of mass destruction. In this article, the current status of research in these areas is reviewed.
Greenfield RA, Bronze MS. (2004). Current therapy and the development of therapeutic options for the treatment of diseases due to bacterial agents of potential biowarfare and bioterrorism. Current Opinion in Investigational Drugs. 5: 2, 135-140.	An important part of biodefense is the optimization of current therapy and the development of new therapeutic options for the treatment of the diseases most likely encountered in the form of biological weapons. Guidelines for the prevention and treatment of anthrax, plague, tularaemia and botulinum toxin intoxication are reviewed. The strategies in development for the prevention of anthrax focus primarily on active and passive immunization against protective antigen, because of its central role as a toxin delivery module. Novel vaccine strategies for plague, tularaemia and botulism are also reviewed.
Greenfield WS (1880). Further investigations on anthrax and allied diseases in man and animals. Lancet 116: 965-966	Vaccination of cow with B anthracis from guinea pig.
Greenough PR. (1965). Anthrax and antibiotics. Vet Rec 77: 784-785	Outbreak in dairy cattle. Pencillin treatment led to rapid recovery in many cattle. Diagnosis of anthrax in animals that had been treated and died was more difficult. Incubation period from exposure to disease was consistently 3-4 days.
Grohs P, Podglajen I, Gutmann L. (2004). Activities of different fluoroquinolones	Service de Microbiologie, Hopital Europeen Georges Pompidou, Paris, France. Three sets of mutants of *Bacillus anthracis* resistant to fluoroquinolones were selected on ciprofloxacin and moxifloxacin

against *Bacillus anthracis* mutants selected in vitro and harboring topoisomerase mutations. Antimicrob Agents Chemother. 48(8):3024-7.	in a stepwise manner from a nalidixic acid-resistant but fluoroquinolone-susceptible plasmidless strain harboring a Ser85Leu GyrA mutation. A high level of resistance to fluoroquinolones could be obtained in four or five selection steps. In each case, ParC was the secondary target. However, in addition to the GyrA mutation, expression of high-level resistance required (i) in the first set of mutants, active drug efflux associated with a mutation in the QRDR of ParC; (ii) in the second set, two mutations in the QRDR of ParC associated with a mutation in GyrB; and (iii) in the third set, two QRDR mutations, one in ParC and one in GyrA. Interestingly, several selection steps occurred without obvious mutations in the QRDR of any topoisomerase, thereby implying the existence of other resistance mechanisms. Among the fluoroquinolones tested, garenoxacin showed the best activity.
Gryadunov DA, Mikhailovich VM, Noskov AN, Lapa SA, Sobolev A Yu, Pan'kov SV, Rubina A Yu, Zasedatelev AS, Mirzabekov AD. (2001). Detection of *Bacillus anthracis* using multiplex PCR on the oligonucleotide biochip. Doklady Biochemistry and Biophysics. 381: 1/6, 384-386.	In this study, a biochip was designed that allowed the differentiation of B. anthracis from other Bacillus strains using multiplex polymerase chain reaction (PCR). The design and structure of the biochip is described, including the synthesis of primers. Results indicate that this method provides an opportunity to reliably detect pathogenic strains of B. anthracis due to the substantial number and functional importance of the target sequences. Combining the specific and allele-specific primers in the multiplex PCR permits simultaneous detection of the genes encoding diagnostically important proteins and minor polymorphisms in the genes controlling the ribosomal RNA synthesis. This approach is useful in dealing with either a genetically engineered strain that does not belong to bacilli but produces toxic substances or with vaccine strains lacking a capsule.
Guarner J, Jernigan JA, Shieh WJ, et al. (2003). Pathology and pathogenesis of bioterrorism-related inhalational anthrax. Am J Pathol 163: 701–9.	[During et al 2001, 4]
Guidi-Rontani C, Levy M, Ohayon H, Mock M. (2001). Fate of germinated *Bacillus anthracis* spores in primary murine macrophages. Molecular Microbiology. . 42: 4, 931-938.	We investigated the fate of germinated *Bacillus anthracis* spores after their germination in Swiss murine peritoneal macrophages and in the cell line RAW264.7. We found that the lethal toxin and the oedema toxin are germ-associated factors that are essential for the survival of the vegetative form in host cells. We also found that pX02 is not involved in this complex pathogenic process. By transmission electron microscopy, we showed the tight interaction between the exosporium of the spore and the phagosomal membrane of the macrophage. Our data strongly suggest that the B. anthracis toxinogenic, unencapsulated Sterne strain (7702) does not multiply within macrophages. These results contributed to reveal the strategies used by B. anthracis to survive within the host and to reach the external medium where they proliferate.
Guidi-Rotani C (2002). The alveolar macrophage: the trojan horse of Bacillus anthracis. Trends Microbiol 10: 405–409	[Cote et al 2005]
Guidi-Rotani C, Weber-Levy M, Labruyere E, Mock M. (1999). Germination of Bacillus anthracis spores within alveolar macrophages. Mol Microbiol 31: 9–17	[Cote et al 2005]
Guo MQ, Zhu GX. (1984). Experimental report on the production of anthrax spore vaccines using aerated soyo bean sprouts broth as medium. Journal of Veterinary Science and Technology (Shouyi Keji Zazhi).. 5, 17-22.	[Chinese]

Gyssens IC, Weyns D, Kullberg BJ, Ursi JP. (2001). A patient in Belgium with cutaneous anthrax. Nederlands Tijdschrift voor Geneeskunde.. 145: 49, 2386-2388.	A 23-year-old Turkish woman with an infection of the left thumb was admitted in a hospital in Belgium. The clinical picture was typical for cutaneous anthrax. Microbiological tests confirmed *Bacillus anthracis*' infection. She recovered when treated with penicillin, although later tests revealed that the bacteria were resistant to this antibiotic. The patient became infected as a result of wounding herself with the teeth of an illegally slaughtered sheep, which had possibly become infected in the pasture. Recognizing the characteristic clinical picture of cutaneous anthrax is essential for prompt treatment and a favourable prognosis.
Hallis B, Silman NJ, Noonan S, Baker R, Roberts AD, Quinn CP, Marks T, Wiblin C, Lloyd G, Robinson A, Hudson MJ. (2002). Novel in vitro functional assays for the determination of anthrax toxin components. Dev Biol (Basel). 111: 321-326	Centre for Applied Microbiology and Research, Salisbury, UK. bassam.hallis@camr.org.uk The characterisation and evaluation of the UK licensed human anthrax vaccine depends on several in vivo tests that determine its safety and potency. Assays for the determination of functionally active and/or immunoreactive toxin components and S-layer proteins have been developed and applied to the characterisation of anthrax vaccine. These technologies may support production of consistent and effective vaccines, and may ultimately reduce the requirements for in vivo testing.
Halsey NA. (2002). Anthrax vaccine and causality assessment from individual case reports. Pharmacoepidemiol Drug Saf. 11(3): 185-187	Discussion 203-204. Comment in: Pharmacoepidemiol Drug Saf. 2002 Oct- 11(7):615-6.
Halverson KM, Panchal RG, Nguyen T, Gussio R, LittleSF, Misakian M, Bavari S, Kasianowicz JJ (2005). Anthrax biosensor: protective antigen ion channel asymmetric blockade. J Biol Chem	
Hambleton P, Carman JA, Melling J. (1984). Anthrax: the disease in relation to vaccines. Vaccine 2: 2, 125-132.	
Hambleton P, Turnbull PC. (1990). Anthrax vaccine development: a continuing story. Adv Biotechnol Processes. 13: 105-122	Division of Biologics, Centre for Applied Microbiology and Research, Salisbury, Wiltshire, England.
Hanna P (1998). How anthrax kills. Science 280: 1671, 1673–4.	[During et al 2001, 25]
Hanna P (1999). Lethal toxin actions and their consequences. J Appl Microbiol 87: 285-287	
Hanna PC, Acosta D, Collier RJ. (1993). On the Role of Macrophages in Anthrax. PNAS 90: 10198-10201.	
Hanna PC, Ireland JA. (1999). Understanding Bacillus anthracis pathogenesis. Trends Microbiol 7: 180–182	[Cote et al 2005]

Hanson RP (1959) The earliest account of anthrax in man and animals in North America J Am Vet Med Assoc 135: 463-465	1824: Kercheval describes an outbreak of anthrax in cattle and infection of four farmers who handled the dead carcasses.
Hardjoutomo S, Poerwadikarta MB, Patten BE, Barkah K. (1993). The application of ELISA to monitor the vaccinal response of anthrax vaccinated ruminants. Penyakit Hewan.. 25: 46A, 7-10.	Groups of 32 sheep, 16 goats and 32 beef cattle raised on confined open grazing land were vaccinated with anthrax spore vaccine at a dose of 1 ml per head of cattle and 0.5 ml per head of sheep and goats s.c.. A second dose of vaccine was given 8 weeks after the initial vaccination to 1 group of the cattle and sheep but not to the goats. All the animals were bled regularly at 2-4 week intervals and sera was tested by an anthrax antibody ELISA. The optical density was converted to ELISA units (EU) and used to express the antibody level in the vaccinated animals. The results of the antibody ELISA indicated that vaccinated ruminants have higher EU values than unvaccinated animals. All experimental animals responded to the initial vaccination with antibodies lasting for 12 weeks post vaccination. Animals receiving the booster injection showed a marked rise in antibody response within 2 weeks of vaccination, the antibody then gradually decreased thereafter.
Harris-Smith P, Smith H, Keppie J (1958). Production in vitro of the toxin of *Bacillus anthracis* previously recognised in vivo. J Gen Microbiol 19: 91-103	'… only by continuous culture have the organisms been kept in their toxigenic growth phase.'
Hartley HA, Baeumner AJ. (2003). Biosensor for the specific detection of a single viable B. anthracis spore. Analytical and Bioanalytical Chemistry. 376: 3, 319-327.	A simple membrane strip-based biosensor for the detection of viable B. anthracis spores was developed and combined with a spore germination procedure as well as a nucleic acid amplification reaction to identify as little as one viable B. anthracis spore in less than 12 h. The biosensor is based on identification of a unique mRNA sequence from the anthrax toxin activator (atxA) gene encoded on the toxin plasmid, pXO1. Preliminary work relied on plasmid vectors in both E. coli and B. thuringiensis expressing the atxA gene. Once the principle was firmly established, the vaccine strain of B. anthracis was used. After inducing germination and outgrowth of spores of B. anthracis (Sterne strain), RNA was extracted from lysed cells, amplified using nucleic acid sequence-based amplification (NASBA), and rapidly identified by the biosensor. While the biosensor assay requires only 15-min assay time, the overall process takes 12 h for the detection of as little as one viable B. anthracis spore, and is shortened significantly, if larger amounts of spores are present. The biosensor is based on an oligonucleotide sandwich-hybridization assay format. It uses a membrane flow-through system with an immobilized oligonucleotide probe that hybridizes with the target sequence. Signal amplification is provided when the target sequence hybridizes to a second oligonucleotide probe that has been coupled to dye-encapsulating liposomes. The dye in the liposomes then provides a signal that can be read visually or quantified with a hand-held reflectometer. The biosensor can detect as little as 1.5 fmol of target mRNA. Specificity analysis revealed no crossreactivity with closely related species such as B. cereus, B. megaterium, B. subtilis, B. thuringiensis etc.
Hedlund KW. (1992). Anthrax toxin: history and recent advances and perspectives. Journal of Toxicology, Toxin Reviews.. 11: 1, 41-88.	
Heeren, RH. (1947). Anthrax in Louisiana. New Orleans Medical and Surgical	

Journal 99: 545,	
Heijne G von (2005). Translocation of anthrax toxin: Lord of the Rings. Science 309: 709-710	
Henry M. (1922). The incidence of anthrax in stock in Australia. J Proc R Soc NSW 56: 44-61	'On the part of the farmer and stockowner, action is only required on two matters to bring about the desired result, prompt notification of mortality which might be due to anthrax, and destruction by fire of all carcases of animals dead of anthrax.'
Henry M. (1936). A further note on the incidence of anthrax in stock in Australia. Aust Vet J 12: 235	
Hering D, Thompson W, Hewetson J, Little S, Norris S, Pace-Templeton J. (2004). Validation of the anthrax lethal toxin neutralization assay. Biologicals. 32(1): 17-27	Product Development and Regulatory Affairs, US Army Medical Research Institute of Infectious Diseases 1425 Porter Street, Fort Detrick, Frederick, MD 21702-5011, USA. Donna.Hering@det.amedd.army.mil A validation of the performance characteristics of a toxin neutralization assay is presented. This in vitro assay measures the functional ability of antisera, containing antibodies to anthrax lethal toxin, to specifically protect J774A.1 cells against *Bacillus anthracis* lethal toxin cytotoxicity. This colormetric assay is based upon the reduction of MTT by living cells. Human and rabbit antisera produced against anthrax vaccine absorbed (AVA) were used to validate the assay. Results showed a high level of repeatability and reproducibility, particularly for a bio-assay. Inter-assay variability in absorbance values was the most prominent negative finding however, an acceptable level was demonstrated with a ratio [neutralization ratio (NR)] of the test serum 50% effective dose (ED(50)) to the reference standard ED(50). Accuracy was maintained, even in samples with minimal neutralizing capacity, and linearity was noted when sample dilutions resulted in accurate prediction of the Y(max)and Y(min). Specificity tests demonstrated that normal sera did not have an observable effect on the ability of the reference standard to neutralize toxin. The assay remained stable against time, temperature, and freeze/thaw effects on the reference standards, but not on the toxin. The assay also remained stable against media and solution storage effects. Cell passage number and cell plating density were two critical parameters identified during the robustness studies that may be responsible for inter-assay variability in absorbance values. The work was performed in accordance with the FDA's Bioanalytical Method Validation Guidance for Industry and the FDA's Good Laboratory Practice for Nonclinical Laboratory Studies (21 CFR Part 58).
Hermanson G, Whitlow V, Parker S, Tonsky K, Rusalov D, Ferrari M, Lalor P, Komai M, Mere R, Bell M, Brenneman K, Mateczun A, Evans T, Kaslow D, Galloway D, Hobart P. (2004). A cationic lipid-formulated plasmid DNA vaccine confers sustained antibody-mediated protection against aerosolized anthrax spores. Proc Natl Acad Sci U S A. 101(37): 13601-13606	Vical Inc., San Diego, CA 92121, USA. ghermanson@vical.com DNA vaccines provide an attractive technology platform against bioterrorism agents due to their safety record in humans and ease of construction, testing, and manufacture. We have designed monovalent and bivalent anthrax plasmid DNA (pDNA) vaccines encoding genetically detoxified protective antigen (PA) and lethal factor (LF) proteins and tested their immunogenicity and ability to protect rabbits from an aerosolized inhalation spore challenge. Immune responses after two or three injections of cationic lipid-formulated PA, PA plus LF, or LF pDNAs were at least equivalent to two doses of anthrax vaccine adsorbed (AVA). High titers of anti-PA, anti-LF, and neutralizing antibody to lethal toxin (Letx) were achieved in all rabbits. Eight or nine animals in each group were challenged with 100x LD(50) of aerosolized anthrax spores 5 or 9 weeks after vaccination. An additional 10 animals vaccinated with PA pDNA were challenged >7 months postvaccination. All animals receiving PA or PA plus LF pDNA vaccines were protected. In addition, 5 of 9 animals receiving LF pDNA survived, and the time to death was significantly delayed in the others. Groups receiving three immunizations with PA or PA plus LF pDNA showed no increase in anti-PA, anti-LF, or Letx neutralizing antibody titers postchallenge, suggesting little or no spore germination. In contrast, titer increases were seen in AVA animals, and in surviving animals vaccinated with LF pDNA alone. Preclinical evaluation of this cationic lipid-formulated bivalent

	PA and LF vaccine is complete, and the vaccine has received U.S. Food and Drug Administration Investigational New Drug allowance.
Heymann DL (editor) (2004). Anthrax. *In* Control of Communicable Diseases Manual, 18th Edition, American Public Health Association, Washington DC, pp 20-25	Identification, causative agent, occurrence, reservoir, mode of transmission, incubation period, period of communicability, susceptibility, methods of control,
Hicks RP, Bhattacharjee AK, Koser BW, Traficante DD. (2004). The anthrax protective antigen (PA_{63}) bound conformation of a peptide inhibitor of the binding of lethal factor to PA_{63}: as determined by trnoesy nmr and molecular modelling. J Med Chem 47: 5347-5355.	
Hicks RP, Hartell MG, Nichols DA, Bhattacharjee AK, van Hamont JE, Skillman DR. (2005). The medicinal chemistry of botulinum, ricin and anthrax toxins. Curr Med Chem. 12(6):667-90.	Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC 20307-5100, USA. Rickey.Hicks@NA.AMEDD.ARMY.MIL The potential use of weapons of mass destruction (nuclear, biological or chemical) by terrorist organizations represents a major threat to world peace and safety. Only a limited number of vaccines are available to protect the general population from the medical consequences of these weapons. In addition there are major health concerns associated with a pre-exposure mass vaccination of the general population. To reduce or eliminate the impact of these terrible threats, new drugs must be developed to safely treat individuals exposed to these agents. A review of all therapeutic agents under development for the treatment of the illnesses and injuries that result from exposure to nuclear, biological or chemical warfare agents is beyond the scope of any single article. The intent here is to provide a focused review for medicinal and organic chemists of three widely discussed and easily deployed biological warfare agents, botulinum neurotoxin and ricin toxins and the bacteria *Bacillus anthracis*. Anthrax will be addressed because of its similarity in both structure and mechanism of catalytic activity with botulinum toxin. The common feature of these three agents is that they exhibit their biological activity via toxin enzymatic hydrolysis of a specific bond in their respective substrate molecules. A brief introduction to the history of each of the biological warfare agents is presented followed by a discussion on the mechanisms of action of each at the molecular level, and a review of current potential inhibitors under investigation.
Hindson BJ, Brown SB, Marshall GD, McBride MT, Makarewicz AJ, Gutierrez DM, Wolcptt DL, Metz TR, Madabhushi RS, Dzenitis JM, Colston BW. (2004). Development of an automated sample preparation module for environmental monitoring of biowarfare agents. Anal Chem 76: 3492-3497	An automated sample preparation module, based upon sequential injection analysis (SIA), has been developed for use within an autonomous pathogen detection system. The SIA system interfaced aerosol sampling with multiplexed microsphere immunoassay-flow cytometric detection. Metering and sequestering of microspheres using SIA was found to be reproducible and reliable, over 24-h periods of autonomous operation. Four inbuilt immunoassay controls showed excellent immunoassay and system stability over five days of unattended continuous operation. Titration curves for two biological warfare agents, *Bacillus anthracis* and Yersinia pestis, obtained using the automated SIA procedure were shown to be similar to those generated using a manual microtiter plate procedure.
Hinman AR (2005). Financing vaccines in the 21st Century. Recommendations from	

the National Vaccine Advisory Committee. Am J Prev Med 29: 71-75	
Hoffmaster AR, Koehler TM. (1999). Control of virulence gene expression in *Bacillus anthracis*. J Appl Microbiol 87: 279-281	
Hoffmaster AR, Meyer RF, Bowen MP, Marston CK, Weyant RS, Barnett GA, Sejvar JJ, Jernigan JA, Perkins BA, Popovic T (2002). Evaluation and Validation of a Real-Time Polymerase Chain Reaction Assay for Rapid Identification of *Bacillus anthracis*. Emerg Infect Dis 8: 1178-1182	
Holmes RK. (2001). Anthrax. In Harrison's Principles of Internal Medicine, 15th edition, edited by E Braunwald et al, McGraw-Hill, New York, p 914-915	
Horne AD, Clifford J, Goldenthal KL, Kleppinger C, Lachenbruch PA (2004). Preventive vaccines against bioterrorism: evalution of efficacy and safety. Baccine 23: 84-90	
Hsu VP, Lukacs SL, Handzel T, Hayslett J, Harper S, Hales T, Semenova VA, Romero-Steiner S, Elie C, Quinn CP, Khabbaz R, Khan AS, Martin G, Eisold J, Schuchat A, Hajjeh RA. (2002). Opening a *Bacillus anthracis*-containing envelope, Capitol Hill, Washington, D.C.: The Public Health Response. Emerg Infect Dis 8 (10), 1039– 1043.	
Huchzermeyer FW. (1997). Animal health risks associated with ostrich products. Rev Sci Tech. 16(1):111-6.	Onderstepoort Veterinary Institute, Onderstepoort, South Africa. Five diseases recorded in ostriches are regarded as posing a potential animal health threat to meat-importing countries. Newcastle disease causes an atypically low mortality in ostriches: infected birds display typical nervous symptoms but no pathognomonic lesions which could be detected during post-mortem inspection. The vaccination of feedlot birds and a thorough ante-mortem examination are regarded as necessary precautions to ensure virus carriers are not among those animals destined for slaughter and subsequent export. Avian influenza produces clinical depression and lesions can be detected at post-mortem examination. Borna disease appears to affect mainly younger birds, and the virus is probably not present in the meat of affected birds. Finally, there is little evidence to suggest that ostriches could play a

	role in the epidemiology of transmissible spongiform encephalopathies. Cases of anthrax are extremely rare. The importation of deboned ostrich meat reduces the risk of infected scraps being fed to susceptible animals.
Hughes JM, Gerberding JL. (2002). Anthrax bioterrorism: lessons learned and future directions. Emerg. Infect. Dis. 8 (10), 1013–1014.	[CDC rapid response teams]
Hughes KL. (1991). History of veterinary public health in Australasia. Rev Sci Tech. 10(4): 1019-1040	Veterinary Science Faculty, University of Queensland, Australia. The geographic isolation of Australasia has played a significant role in preventing the introduction of exotic diseases or in limiting the spread of many diseases which entered after settlement. Some infections such as psoroptic mange, tuberculosis and brucellosis became widely dispersed and some were ultimately to require novel methods to curtail them, e.g. greater use of rail and road transportation to convey stock, improved methods to locate and muster livestock in bush terrain (helicopters), improved diagnostic tests and the introduction of effective methods for tracing diseases found at abattoirs to the farms of origin. From the 1860s to the 1880s, there were such high mortalities from anthrax in Australia that a business syndicate associated with the Pasteur Institute established a laboratory in Sydney to produce anthrax vaccine from 1890 to 1898. The two-dose vaccine developed by Pasteur was unable to compete with a single dose spore vaccine later pioneered locally by Gunn and McGarvie-Smith. The most important achievements in veterinary public health in Australasia have been the successful eradication of brucellosis, the virtual eradication of hydatid disease in New Zealand and Tasmania, the substantial progress made in the eradication of tuberculosis from all but small regions of Australasia, and the development of a commercial vaccine to prevent Q fever in humans.
Hugh-Jones M (1998). 1996-1997 global anthrax report. J Appl Microbiol 87: 189-191	“One of the epidemiological tragedies was Pasteur’s public experiment at Pouilly le-Fort where the resulting massive sporulation instituted the belief in permanent ground contamination. This belief has too often prevented the thorough investigation of the sources of outbreaks. It is too easy to ascribe a case of anthrax to a previous death on the same farm in a previous decade, however distant,on the slimmest of evidence or even without any evidence. The reality is that soil is dangerous for a limited period; many years ago Max Sterne defined it as ‘three months to three years’. Experience shows that he was correct. This is not to deny the existence of champs maudit and contaminated burial sites, but these are rare. If they were common, how did Cyprus successfully eradicate anthrax. Anthrax was long documented in Jamaica and in New Zealand where it has not been known in decades.”
Hugh-Jones ME (1996). World situation 1993–94. In: Anthrax. Proceedings of an International Workshop, Winchester, 19–21 September 1995 (PCB Turnbull, ed). Salisbury Medical Bulletin, Special Supplement 87:1–2.	
Hugh-Jones ME, de Vos V (2002). Anthrax and wildlife. Revue Scientifique et Technique - Office International des Epizooties.. 21: 2, 359-383.	Kangaroos and dingos susceptible Age susceptibility – older more susceptible than younger Outbreak pattern – the disease passes through the population at risk with most mortalities on a wave-like front with a high incidence of new cases occurring at the leading edge. Behind the front the number of cases progressively decreases and after a few weeks only sporadic isolated mortalities are seen. Etosha National Park – contamination occurred with 1:20 dead antelope and even then only 5% of contaminated sites had significant contamination.

	Carrier state in recovered pigs possible. Treatment of carcases with 5% formaldehyde stops carcase scavenging and disinfects the environment. Burial is not considered a long-term solution and can result in future outbreaks. Urgent need for a cheap and highly effective oral vaccine
Hugh-Jones ME, Hussaini SN. (1974). An anthrax outbreak in Berkshire. Vet Rec 94: 228-232	Primary case – possible exposure to old burial site. Secondary cases – contamination from primary case. Soil consumption.
Hugh-Jones ME, Hussaini SN. (1975). Anthrax in England and Wales, 1963-1972. Vet Rec 97: 256-261	Contaminated feed a major source of exposure. Young cattle rarely affected by anthrax.
Hugh-Jones ME, Turnbull PCB, Jones MN, Hutson RA, Quinn CP, Kramer JM. (1991). Re-examination of the mineral supplement associated with a 1972 anthrax outbreak. Veterinary Record.. 128: 26, 615-616.	Two specimens of calcined magnesite that had been used as a mineral supplement at the institute where the outbreak had occurred were examined. Large numbers of *Bacillus anthracis*-like organisms were isolated from dilutions of the untreated, heated and alcohol shocked samples. The bacteria became increasingly obvious in successive dilutions of the suspensions of the specimens. It was estimated that there were 200 000 B. anthracis spores/g of calcined magnesite. Four mice inoculated with suspensions of the bacteria suffered no ill effects. On the basis of typical bacterial and colonial morphology and biochemical tests 2 randomly selected colonies (S1 and S2) were identified as B. anthracis. Plasmid analysis and DNA probe studies showed that one of the isolates (S1) carried the genes for the production of anthrax toxin but neither isolate carried the genes controlling the elaboration of the capsule. A guineapig injected with a suspension of the S1 isolate showed no ill effects and did not develop detectable anti-protective antigen antibodies nor protection. The results suggest that the S1 and S2 isolates are tox+/cap- and tox-/cap- variants of B. anthracis; the possibility that they represent a virulent parent (tox+/cap+) B. anthracis which over time has lost the pX01 or pX02 plasmids is discussed.
Hull AK, Criscuolo CJ, Mett V, Groen H, Steeman W, Westra H, Chapman G, Legutki B, Baillie L, Yusibov V. (2005). Human-derived, plant-produced monoclonal antibody for the treatment of anthrax. Vaccine.. 23: 17/18, 2082-2086.	The unpredictable nature of bio-terrorism compels us to develop medical countermeasures that will enable authorities to treat individuals exposed to agents such as anthrax. We report the feasibility of producing a protective, human-derived, monoclonal antibody directed against the protective antigen (PA) of *Bacillus anthracis* in plants. This was achieved by transient expression using agroinfiltration of Nicotiana benthamiana plants. The resulting antibody was able to neutralize toxin activity in vitro and in vivo at a comparable level to that seen for its hybridoma-produced counterpart.
Hussaini SN, Ruby KR. (1976). Sporicidal activity of peracetic acid against B. anthracis spores. Veterinary Record.. 98: 13, 257-259.	A complete sporicidal effect was observed at a concentration of 3%. This concentration is recommended for the treatment of infected pastures and soil.
Hutson RA, Duggleby CJ, Lowe JR, Manchee RJ, Turnbull PCB. (1993). The development and assessment of DNA and oligonucleotide probes for the specific detection of *Bacillus anthracis*. Journal of Applied Bacteriology.. 75: 5, 463-472.	Two DNA probes and a number of oligonucleotide probes were designed from the virulence factor genes of B. anthracis and tested for specificity against 52 B. anthracis strains and 233 Bacillus strains encompassing 23 other species. A rapid slot blotting technique was used for screening the large numbers of isolates involved. All probes tested appeared to be specific for B. anthracis under high stringency conditions. These probes could differentiate between virulent and avirulent strains. The probes were also applied to the detection of B. anthracis in routine environmental and clinical samples. A non-radioactive hybridization and detection system based on digoxigenin-11-dUTP was developed.
Huxsoll DL, Patrick WC, Parrott CD (1987).	

Veterinary services in biological disasters. JAVMA 190: 714-722	
Iacono-Connors LC, Welkos SL, Ivins BE, Dalrymple JM. (1991). Protection against anthrax with recombinant virus-expressed protective antigen in experimental animals. Infection and Immunity.. 59: 6, 1961-1965.	The antigenicity of the products obtained by cloning and expression of the protective antigen (PA) gene of *Bacillus anthracis* in both vaccinia and a baculovirus was characterized. PA expressed by the recombinant vaccinia viruses elicited a partial protective immune response against a lethal B. anthracis spore challenge in guineapigs and mice. The WR strain vaccinia virus recombinant (WR-PA) protected 60% of male mice and 50% of guineapigs. WR-PA elicited high anti-PA antibody titres in mice but not in guineapigs. Connaught strain vaccinia virus recombinants failed to protect any immunized animals. PA purified from baculovirus recombinant-infected cultures plus adjuvant partially protected male CBA/J mice and completely protected female Hartley guineapigs from challenge. Both the recombinant and non-recombinant PA preparations combined with adjuvant elicited high anti-PA antibody titres in Hartley guineapigs and CBA/J mice. These results demonstrate that the recombinant baculovirus- and vaccinia virus-produced PAs were immunogenic in both guineapigs and mice that the baculovirus-PA recombinant was a useful source of immunogenic PA, and that vaccinia virus-PA recombinants may be feasible live anthrax vaccine candidates worthy of consideration for further development as live vaccines.
Ibrahim KH, Brown G, Wright DH, Rotschafer JC. (1999). *Bacillus anthracis*: medical issues of biologic warfare. Pharmacotherapy 19(6): 690-701	College of Pharmacy, University of Minnesota, Minneapolis, USA. Recent world events refocused attention on the possibility of nations engaging in biologic warfare, including an attack with *Bacillus anthracis*. The single available anthrax vaccine in the United States for human use, formerly known as MDPH-PA, has decreased ability to protect laboratory animals against virulent B. anthracis strains, especially compared with new vaccines being developed. Studies with these vaccines, however, have several shortcomings. The pathogenesis, diagnosis, treatment, and prophylaxis of anthrax are discussed, as well as the implications that an attack with B. anthracis would place on the health care system.
Idrisov GZ. (1976). Morphological evaluation of the efficacy and immunological competition of antigens from an associated vaccine of Brucella strain 19 and *Bacillus anthracis* strain STI. Uchenye Zapiski Kazanskogo Veterinarnogo Instituta.. 122: 118-123.	Thirty-seven sheep were given an associated aerosol vaccine against Brucella abortus strain 19 and *Bacillus anthracis* strain STI (Russian vaccine strain). The vaccine provided strong immunity against virulent Brucella. Immunomorphological changes included an activation of reticuloendothelial cells in the lungs, with proliferation of plasmocytes and increased quantities of IgA. Competition on macrophage receptors and on the predecessors of antibody-forming cells between Brucella and B. anthracis antigens did not have a marked effect on the appearance of immunological reactions in the organs to each antigen.
Il'yasov B. (1976). Indirect haemagglutination tests on cattle immunized simultaneously against anthrax, brucellosis and blackleg. Izvestiya Akademii Nauk Kazakhskoi SSR, Seriya Biologicheskaya.. 2, 81-82, 92.	Separate antigens prepared from *Bacillus anthracis*, Clostridium chauvoei and Brucella abortus were used to sensitize sheep erythrocytes. These erythrocytes were used in the indirect haemagglutination test on serum from cattle immunized with monovalent or trivalent vaccines between 5 and 60 days beforehand. The tests were sensitive and specific. Peak antibody titres against brucellosis in five groups of five calves were 1:40-1:98 on the 12th day after vaccination; in the case of anthrax 1:88-1:132 on the 20th day; in the case of blackleg 1:96-1:136 on the 20th day.
Inglesby TV (1999). Anthrax: a possible case history. Emerg Infect Dis 5: 556-560	Fictitious case of aerosol release of anthrax spores at profession football match. 20,000 persons infected, 4,000 deaths, most in first 10 days after attack. 250,000 persons received antibiotics.
Inglesby TV, O'Toole T, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Friedlander AM, Gerberding J, Hauer J,	Erratum in: JAMA 2002 Oct 16;288(15):1849. Comment in: JAMA. 2002 Oct 16;288(15):1848-9; author reply 1848-9. JAMA. 2002 Oct 16;288(15):1848; author reply 1848-9. Johns Hopkins Center for Civilian Biodefense Strategies, Johns Hopkins University, Baltimore, MD 21202, USA.

Hughes J, McDade J, Osterholm MT, Parker G, Perl TM, Russell PK, Tonat K; Working Group on Civilian Biodefense. (2002). Anthrax as a biological weapon, 2002: updated recommendations for management. JAMA. 287(17): 2236-2252.	tvi@jhsph.edu OBJECTIVE: To review and update consensus-based recommendations for medical and public health professionals following a *Bacillus anthracis* attack against a civilian population. PARTICIPANTS: The working group included 23 experts from academic medical centers, research organizations, and governmental, military, public health, and emergency management institutions and agencies. EVIDENCE: MEDLINE databases were searched from January 1966 to January 2002, using the Medical Subject Headings anthrax, *Bacillus anthracis*, biological weapon, biological terrorism, biological warfare, and biowarfare. Reference review identified work published before 1966. Participants identified unpublished sources. CONSENSUS PROCESS: The first draft synthesized the gathered information. Written comments were incorporated into subsequent drafts. The final statement incorporated all relevant evidence from the search along with consensus recommendations. CONCLUSIONS: Specific recommendations include diagnosis of anthrax infection, indications for vaccination, therapy, postexposure prophylaxis, decontamination of the environment, and suggested research. This revised consensus statement presents new information based on the analysis of the anthrax attacks of 2001, including developments in the investigation of the anthrax attacks of 2001; important symptoms, signs, and laboratory studies; new diagnostic clues that may help future recognition of this disease; current anthrax vaccine information; updated antibiotic therapeutic considerations; and judgments about environmental surveillance and decontamination.
Institute of Medicine (IOM) (2002). Joellenbeck LM, Zwanziger L, Durch JS, Strom BL, editors. The Anthrax Vaccine: Is it Safe? Does it Work? Washington, DC: National Academy Press, April 2002, xxi + 265 pp.	
Ipatenko NG (1979). Special features of anthrax diagnosis in swine. Veterinariya, Moscow. 12, 36-39.	Microscopic examination of smears and bacteriological investigation of 2173 lymph nodes and 380 internal organs from swine revealed *Bacillus anthracis* in 559 cases. Detailed examination of 208 carcasses indicated local development of the disease, mainly in the head region; only 15 cases involved other organs - mesenteric lymph nodes (8), spleen (4), kidneys (one), root of tongue (one) and cartilage of the larynx (one). Bilateral infection of the submaxillary lymph nodes was found in 40% of infected carcasses, and unilateral in 21%. Eighteen carcasses showed infection of the caudal lymph nodes (ventral, medial and dorsal), 27 of the tonsils, and 32 of the retropharyngeal lymph nodes. Latent or chronic forms of the disease were dominant. Listlessness was a typical symptom of all forms, and the presence of subcutaneous carbuncles. Typical of the chronic form were a temporary rise in temperature, periadenitis and periodic oedema of the neck. Death can occur through swelling of the larynx and suffocation. Virulent bacilli are found only in nodes drawing lymph from infected parts of the intestines. In the intestinal forms, mainly affected are Peyer's patches, becoming necrotic, with a greenish-yellow or yellowish-grey crumbly mass, which tears away to reveal ulcers. In all forms there is frequent infarction. The acute form is readily diagnosable, as the symptoms are pronounced, and death ensues within 2-3 days. Differential diagnosis should include malignant oedema, salmonellosis, and haemorrhagic septicaemia.
Ipatenko NG (1980). Antibiotics in anthrax. Veterinariya, Moscow 7, 31-32.	Penicillin 41464, streptomycin 81064, biomycin 21264 and dibiomycin all proved effective against *Bacillus anthracis* in vitro, but dibiomycin less so. At first 112 cattle were injected too late with penicillin and died, but injection of 5000 units penicillin/kg bodyweight into 224 cattle three times each day at 4-hour intervals for 3 days proved 100% successful. 50 other animals infected with B. anthracis all died without treatment, but 508 treated with penicillin recovered. In swine

	anthrax normally occurs as a local pathological process and rarely as septicaemia. Use of 3000 EU penicillin/kg in combination with 250 ml serum twice daily for 3 days was more effective than injection of penicillin alone; in severe cases the penicillin dose was increased to 5000 EU/kg thrice daily.
Ipatenko NG (1991). Diagnosis of atypical forms of anthrax in pigs. Veterinariya (Moskva).. 1, 38-41.	Bacteriological testing of lymph nodes from 4916 pigs from districts where anthrax occurred, slaughtered at one of 8 abattoirs between 1955 and 1966, yielded 191 isolates of *Bacillus anthracis*. Examination by microscopy, culture and animal inoculation of 2173 suspect lymph nodes and 380 suspect internal organs detected 558 cases of anthrax. It was necessary for meat inspectors to be aware of the pathological changes of anthrax in lymph nodes, tonsils and internal organs (particularly spleen and kidney), and to make use of bacteriological testing.
Ipatenko NG, Gushchin VN, Grigorov VI. (1986). Anthrax: examination of livestock before slaughter and diagnosis at meat inspection. Veterinariya, Moscow. 1, 31-35.	
Ipatenko NG, Manichev AA, Salenko LS, Gavrilov VA, Stepanova VV, Shmorgun BI, Khurai RYa, Bakhtarov SI, Saiitkulov BS, Muryi AA, Abdurashitov TA. (1993). Immunogenicity of Strain 55 anthrax vaccine. Veterinariya (Moskva).. 3, 17-19.	Naturally attenuated strain 55 of *Bacillus anthracis*, isolated from a pig in 1963, provided a satisfactory alternative to the STI strain, used in Russia for the past 45 years. A single injection protected cattle and sheep for a year.
Ipatenko NG, Shchenev AI, Antonyuk VP, Melikhov AD. (1984). Prophylaxis of anthrax. Veterinariya, Moscow.. 8, 27-28.	Analysis of data for the past 30-50 years showed that anthrax occurred once on half the infected premises of a given region, twice in a quarter, three times in 6% and four or more times in 18%. In herds where the disease had occurred 3 times or more, vaccination (with STI spore vaccine) was recommended in spring and autumn.
Ipatenko NG, Tatarintsev NI, Manichev AA, Gushchin VN. (1989). Prophylaxis of anthrax. Veterinariya, Moscow.. 5, 41-42.	
Ipatenko NG, Tatarintsev NT, Manichev AA, Sedov VA, Gushchin VN, Revazov AL, Gutiev AV, Gutiev AN, Bakhtarov SI, Krutskikh VA, Kiselev YuT, Muryi AA, Grigorov VI. (1989). Results of using anthrax vaccine prepared from strain 55. Veterinariya (Moskva).. 8, 7-10.	Vaccine was prepared from attenuated strain 55 of *Bacillus anthracis* at the All-union Research Institute for Veterinary Virology and Microbiology. It was first tested in the Vladimir region in 1984 on 980 sheep, in comparison with the established "STI" vaccine. All sheep resisted challenge infection up to 18 months after vaccination, compared with only half of those vaccinated with "STI". 49 000 cattle, 2000 sheep, 2000 pigs and 99 horses in the Severo-Osetinskaya ASSR were vaccinated between 1985 and 1988. Further large-scale field trials were conducted in other parts of the USSR between 1984 and 1988. The vaccine proved to be safe, and effective in reducing the number of outbreaks of anthrax after a single injection.
Ipatenko NG, Tatarintsev NT, Sedov VA, Gushchin VN. (1987). Epizootiology of anthrax. Veterinariya, Moscow.. 9, 35-37.	Observations made in the USSR over the past 20 years had shown that soil contamination was important for perpetuating anthrax in farm animals. Since the disease was most prevalent in summer and autumn, immunization (preferably with two doses of vaccine) should be done in spring.

Ivins BE, Ezzell JW, Jemski J et al: (1986). Immunization studies with attenuated strains of *Bacillus anthracis*. Infect Immun 52:454,	
Ivins BE, Fellows PF, Nelson GO. (1994). Efficacy of a standard human anthrax vaccine against *Bacillus anthracis* spore challenge in guinea-pigs. Vaccine. 12(10):872-4.	Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011. The efficacy of an anthrax vaccine licensed for human use, MDPH-PA, was tested in guinea-pigs intramuscularly challenged with 10, 100 or 1000 LD50 of spores from two virulent strains of *Bacillus anthracis*, Vollum 1B and Ames. As demonstrated in other investigations, immunization with MDPH-PA provided better protection against challenge with the Vollum 1B strain than with the Ames strain, although vaccine efficacy against the Ames strain was better than previously reported. Enzyme-linked immunosorbent assay of serum antibody titres to B. anthracis protective antigen showed that there was no significant correlation between survival and antibody titres.
Ivins BE, Fellows PF, Pitt L, Estep J, Farchaus J, Friedlander A, Gibbs P. (1995) Experimental anthrax vaccines: efficacy of adjuvants combined with protective antigen against an aerosol *Bacillus anthracis* spore challenge in guinea pigs. Vaccine. 13(18):1779-84.	Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011, USA. The efficacy of several human anthrax vaccine candidates comprised of different adjuvants together with *Bacillus anthracis* protective antigen (PA) was evaluated in guinea pigs challenged by an aerosol of virulent B. anthracis spores. The most efficacious vaccines tested were formulated with PA plus monophosphoryl lipid A (MPL) in a squalene/lecithin/Tween 80 emulsion (SLT) and PA plus the saponin QS-21. The PA+MPL in SLT vaccine, which was lyophilized and then reconstituted before use, demonstrated strong protective immunogenicity, even after storage for 2 years at 4 degrees C. The MPL component was required for maximum efficacy of the vaccine. Eliminating lyophilization of the vaccine did not diminish its protective efficacy. No significant alteration in efficacy was observed when PA was dialyzed against different buffers before preparation of vaccine. PA+MPL in SLT proved superior in efficacy to the licensed United States human anthrax vaccine in the guinea pig model.
Ivins BE, Fellows PF, Pitt MLM, Estep JE, Welkos SL, Worsham PL, Friedlander AM (1996) Efficacy of a standard human anthrax vaccine against *Bacillus anthracis* aerosol spore challenge in rhesus monkeys. Salisbury Medical Bulletin, Special Supplement no.87: 125-126	
Ivins BE, Pitt ML, Fellows PF, Farchaus JW, Benner GE, Waag DM, Little SF, Anderson GW Jr, Gibbs PH, Friedlander AM. (1998). Comparative efficacy of experimental anthrax vaccine candidates against inhalation anthrax in rhesus macaques. Vaccine. 16(11-12):1141-8.	Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011, USA. bruce_ivins@detrick.army.mil The authors examined the efficacy of *Bacillus anthracis* protective antigen (PA) combined with adjuvants as vaccines against an aerosol challenge of virulent anthrax spores in rhesus macaques. Adjuvants tested included i) aluminum hydroxide (Alhydrogel), ii) saponin QS-21 and iii) monophosphoryl lipid A (MPL) in squalene/lecithin/Tween 80 emulsion (SLT). Animals were immunized once with either 50 micrograms of recombinant PA plus adjuvant, or with Anthrax Vaccine Adsorbed (AVA), the licensed human anthrax vaccine. The serological response to PA was measured by enzyme linked immunosorbent assay. Lymphocyte proliferation and serum neutralization of in vitro lethal toxin cytotoxicity were also assayed. In all vaccine groups, anti-PA IgM and IgG titers peaked at 2 weeks and 4-5 weeks

	postimmunization, respectively. Five weeks postimmunization, animals in all vaccine groups demonstrated PA-specific lymphocyte proliferation and sera that neutralized in vitro cytotoxicity. Six weeks after immunization, the animals were challenged by aerosol with approximately 93 LD50 of virulent anthrax spores. Animals were bled daily for 1 week to monitor bacteremia, and deaths were recorded. Anti-PA ELISA titers in all groups of immunized animals were substantially increased 2 weeks after challenge. One dose of each vaccine provided significant protection (> 90%) against inhalation anthrax in the rhesus macaques.
Ivins BE, Welkos SL (1986). Cloning and expression of the *Bacillus anthracis* protective antigen gene in Bacillus subtilis. Infect Immun 54: 537-542	104
Ivins BE, Welkos SL, Knudson GB, Little SF. (1990). Immunization against anthrax with aromatic compound-dependent (Aro-) mutants of *Bacillus anthracis* and with recombinant strains of Bacillus subtilis that produce anthrax protective antigen. Infection and Immunity.. 58: 2, 303-308.	The safety and efficacy of five prototype, live anthrax vaccines were studied in Hartley guinea pigs and CBA/J and A/J mice. Two of the strains, *Bacillus anthracis* FD111 and FD112, are Aro- mutants derived by Tn916 mutagenesis of B. anthracis UM23-1. B. anthracis PA1 and PA2 contain a recombinant plasmid, pPA101 or pPA102, respectively, that carries the gene from B. anthracis encoding synthesis of protective antigen (PA). The final strain, B. subtilis PA7, was isolated from B. subtilis DB104 transformed with pPA101. All five strains were less virulent in guinea pigs and A/J and CBA/J mice than the toxinogenic, nonencapsulated B. anthracis veterinary vaccine Sterne strain. A/J and CBA/J inbred mice represent strains that are innately susceptible and resistant, respectively, to the Sterne strain. These differences in susceptibility are due to differences in ability to produce complement component 5. In guinea pigs, immunization with PA1 or PA2 vegetative cells of PA7 spores protected <more or =>95% from an intramuscular spore challenge with the virulent, "vaccine-resistant" B. anthracis Ames strain. Strain PA2 vegetative cells and strain PA7 spores were as effective as the Sterne strain in Sterne-resistant CBA/J mice, protecting 70% of the mice from Ames strain spore challenge. Immunization with FD111 or FD112 vegetative cells fully protected guinea pigs from challenge. Immunization with FD111 cells protected up to 100% of CBA/J mice and up to 70% of A/J mice.
Ivins BE, Welkos SL, Little SF et al: (1990). Cloned protective activity and progress in development of improved anthrax vaccines. Salisbury Medical Bulletin, special supplement #68, January p 86	
Ivins BE, Welkos SL, Little SF, Crumrine MH, Nelson GO. (1992). Immunization against anthrax with *Bacillus anthracis* protective antigen combined with adjuvants. Infect Immun 60(2): 662-668	Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011. The protective efficacy of immunization against anthrax with *Bacillus anthracis* protective antigen (PA) combined with different adjuvants was tested in Hartley guinea pigs and CBA/J and A/J mice. Adjuvant components derived from microbial products that were tested included threonyl-muramyl dipeptide (threonyl-MDP); monophosphoryl lipid A (MPL); trehalose dimycolate (TDM); and the delipidated, deproteinized, cell wall skeleton (CWS) from either Mycobacterium phlei or the BCG strain of Mycobacterium bovis. Non-microbially derived adjuvants tested included aluminum hydroxide and the lipid amine CP-20,961. In guinea pigs, all adjuvants and adjuvant mixtures enhanced antibody titers to PA as well as survival after a parenteral challenge of virulent B. anthracis Ames spores. In contrast, PA alone or combined with either aluminum hydroxide or CP-20,961 failed to protect mice. Vaccines containing PA combined with threonyl-MDP or MPL-TDM-CWS protected a majority of female CBA/J mice. Statistical analysis of survival data in the guinea pigs indicated that PA-MPL-CWS and PA-MPL-TDM-CWS were more efficacious than the

	currently licensed human anthrax vaccine.
Ivins BE, Welkos SL. (1988). Recent advances in the development of an improved, human anthrax vaccine. Eur J Epidemiol 4(1): 12-19	Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-5011. Human anthrax vaccines currently licensed in the United States and Western Europe consist of alum-precipitated or aluminum hydroxide-adsorbed supernatant material from fermentor cultures of toxigenic, nonencapsulated strains of *Bacillus anthracis*. These vaccines have several drawbacks, including the need for frequent boosters, the apparent inability to protect adequately against certain strains of B. anthracis, and occasional local reactogenicity. Studies are being undertaken to develop an improved human anthrax vaccine which is safe and efficacious, and which provides long-lasting immunity. Aspects being studied include the identification of antigens and epitopes responsible for eliciting protective immunity, the mechanisms of resistance to anthrax infection, the role of specific antibody in resistance, the differences in immunity elicited by living and chemical vaccines, the potential of new adjuvants to augment immunity, and the feasibility of developing safe vaccine strains having mutationally altered toxin genes. Both living and non-living (chemical) prototype vaccines are being developed and tested.
Ivins BE. (1988). The search for a new-generation human anthrax vaccine. Clinical Immunology Newsletter.. 9: 2, 30-32.	
Ivins BE. (1998). Anthrax vaccines -- how stable is the potency? ASM 98th General Meeting, May 18, , Atlanta	
Jackson FC, Wright GC, Armstrong J (1957). Immunization of cattle against experimental anthrax with alum-precipitated protective antigen or spore vaccine. Am J Vet Res 18: 771-777	Protective antigen and spore vaccine. Two doses of PA vaccine needed. Immunity present at 1month but had declined by 3½ months and barely detectable at 7 months. Challenge per os in unvaccinated animals led to characteristic lesion in small intestine and adjacent mesenteric lymph nodes.
Jackson PJ, Hill KK, Laker MT, Ticknor LO, Keim P. (1999). Genetic comparison of *Bacillus anthracis* and its close relatives using amplified fragment length polymorphism and polymerase chain reaction analysis. J Appl Microbiol 87: 23- 269	
Jaiswal TN, Mittal KR. (1979). Potency testing of anthrax spore vaccine (living) in guinea pigs. Indian Veterinary Journal.. 56: 3, 199-201.	Indian anthrax spore vaccine contained 1.5 million viable spores per ml. Potency test in guinea pigs compared with challenge study in sheep and goats and found to provide similar results using a challenge of 100MLD.
Jaros M, Sima O. (1980). Improvement of the laboratory diagnosis of anthrax and the killing of spores in hides to improve	[Czech]

control measures against the introduction of anthrax from abroad. Sbornik Vedeckych Praci Ustredniho Statniho Veterinarniho Ustavu.. 10, 8-12.	
Jedrzejas MJ. (2002). The structure and function of novel proteins of *Bacillus anthracis* and other spore-forming bacteria: development of novel prophylactic and therapeutic agents. Critical Reviews in Biochemistry and Molecular Biology. 37: 5, 339-373.	The overall goal of this review is to summarize the current body of knowledge about the structure and function of major proteins of *Bacillus anthracis* and/or similar spore-forming organisms. B. anthracis is a key spore-forming biological threat agent, as well as human and animal Gram-positive bacterial pathogen. The structural information described here is limited to approximately the last 5 years. This information is then related to the role of the selected proteins in pathogenesis and in the possible development of novel vaccine and/or other antimicrobial agents against spore-forming organisms, including anthrax, a disease caused by B. anthracis. Among spore-forming bacteria, Bacillus and Clostridium species are the predominant spore-forming bacilli that cause serious diseases. The biochemical properties and mechanism of catalysis of the novel spore germination protease that degrades small, acid-soluble proteins protecting DNA against damage, a cofactor independent phosphoglycerate mutase, NAD+ synthetase, and the three know B. anthracis toxins, protective antigen, lethal factor, and oedema factor are described. The studies described in this work review and unify selected information critical for the prevention of microbial diseases such as anthrax. A strategy for the structure-guided development of new prophylactic and therapeutic agents is discussed.
Jefferson T, Demicheli V, Deeks J, Graves P, Pratt M, Rivetti D. (2005). Vaccines for preventing anthrax. Cochrane Database Syst Rev. (2):CD000975.	Army Medical Directorate 5, Ministry of Defence, Building 21a, Keogh Barracks, Ash Vale, HANTS, UK, GU12 5RR. zorria@epinet.co.uk BACKGROUND: Anthrax is an acute bacterial skin disease which may be fatal. Three anthrax vaccines are commercially available but their comparative effectiveness and safety is not clear. OBJECTIVES: The objective of this review was to assess the effects of human anthrax vaccines in healthy adults and children. SEARCH STRATEGY: We searched the Cochrane Controlled Trials Register, Medline, Embase and the reference lists of articles. We handsearched the journal Vaccine and contacted researchers in the field. SELECTION CRITERIA: Randomised and quasi-randomised trials comparing anthrax vaccines with placebo, vaccines for other diseases or no intervention. DATA COLLECTION AND ANALYSIS: Trial quality assessment and data extraction was conducted independently by the six authors. MAIN RESULTS: Two trials involving 16,052 people were included. Both trials had methodological limitations. Compared to placebo, vaccination was associated with a reduced risk of contracting anthrax (relative risk 0.16, 95% confidence interval 0.07 to 0.35). Compared to placebo, the killed vaccine was associated with a higher incidence and severity of adverse effects (odds ratio 5.15, 95% confidence interval 2.28 to 11.61). Just over 5% of participants in the vaccine group reported adverse effects. The effectiveness of the vaccine does not appear to be influenced by the route of inoculation. REVIEWER'S CONCLUSIONS: Killed anthrax vaccines appear to be effective in reducing the risk of contracting anthrax with a relatively low rate of adverse effects. Further research should be restricted to testing new vaccines only.
Jendrek S, Little SF, Hem S, Mitra G, Giardina S. (2003) Evaluation of the compatibility of a second generation recombinant anthrax vaccine with aluminum-containing adjuvants. Vaccine. 21(21-22):3011-8.	Building 320, SAIC-Frederick Inc., National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702, USA. sjendrek@ncifcrf.gov Recombinant protective antigen (rPA) is the active pharmaceutical ingredient in a second generation anthrax vaccine undergoing pre-clinical evaluation. This rPA vaccine differs from the currently licensed vaccine, anthrax vaccine adsorbed (AVA), in that the sole component is a recombinant form of protective antigen (PA). Unlike AVA the rPA vaccine contains no lethal factor (LF) or edema factor (EF), components of the two bipartite toxins, nor many other *Bacillus anthracis*-related contaminating proteins that are present in AVA. The proposed clinical protocol involves adsorption of the rPA to an aluminum-based adjuvant. The adsorptive characteristics of rPA and two aluminum-

	containing adjuvants were examined in a physiological buffer with and without EDTA. Based on the pI of rPA (pI=5.6) and the zero charge point of aluminum hydroxide adjuvant (11.5) and aluminum phosphate adjuvant (4.5), it was predicted and demonstrated that rPA bound in a more efficient manner to aluminum hydroxide adjuvant than to aluminum phosphate adjuvant in the physiological buffer. Binding of the rPA to the aluminum hydroxide adjuvant was decreased by increasing amounts of phosphate in the buffer. The adsorptive capacity for rPA onto aluminum hydroxide adjuvant in the physiological buffer and in water were calculated to be 0.46 mg rPA/mg aluminum in DPBS and 0.73 mg rPA/mg aluminum in water. This study also demonstrated that upon desorption from the aluminum hydroxide adjuvant the rPA was physically intact and free of detectable aggregates. Further, the eluted material was biologically active in an in vitro cytotoxicity assay. Desorption was only possible after an overnight incubation of 2-8 degrees C and not after a room temperature incubation reflecting increased contact with the aluminum hydroxide adjuvant over time. These data suggest that the interaction between rPA and aluminum hydroxide adjuvant is predominantly electrostatic in character.
Jernigan DB, Raghunathan PL, Bell BP. (2002). Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings. Emerg. Infect. Dis. 8 (10), 1019– 1028.	[From October 2 to November 20 2001, a total of 22 cases of anthrax were identified – including 11 cases of inhalation anthrax (that lead to 5 deaths) and 11 cases of cutaneous anthrax.]
Jernigan JA, Stephens DS, Ashford DA, et al. (2001). Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 7: 933–44.	[During et al 2001, 2]
Jiang FuXing; Mao Qun. (1998). Septicaemic anthrax of pigs. Chinese Journal of Veterinary Medicine.. 24: 1, 31-32.	An outbreak of anthrax occurred in pigs in Wantian Township, Guilin (Guangxi), China in February 1997. Four pigs died. Two people were infected; 1 died and the other recovered. The diagnosis was based on clinical signs, Ascoli precipitation test, microscopic examination and bacterial culture. Disease control measures included a restriction on movement of animals, thorough disinfection and vaccination of all pigs, cattle, sheep and goats.
Johnson AD, Spero L. (1981). Comparison of growth and toxin production in two vaccine strains of *Bacillus anthracis*. Applied and Environmental Microbiology.. 41: 6, 1479-1481.	Two vaccine strains of B. anthracis in a 10-litre fermentor were compared for growth patterns and toxin production. Under identical conditions, the Sterne strain produced all three components of anthrax toxin, whereas strain V770 produced only the protective antigen.
Johnson-Winegar A. (1984). Comparison of enzyme-linked immunosorbent and indirect haemagglutination assays for determining anthrax antibodies. Journal of Clinical Microbiology.. 20: 3, 357-361.	The highly sensitive enzyme-linked immunosorbent assay (ELISA) is the in vogue method of detecting antigens and antibodies to them. The author (from the US Army Medical Research Institute of Infectious Diseases) applied it to detection of antibodies in vaccinees to the protective antigen component of anthrax toxin and compared the method with passive haemagglutination (PHA) which has been in use since 1971.
Jones ME, Goguen J, Critchley IA, Draghi DC, Karlowsky JA, Sahm DF, Porschen R, Patra G, DelVecchio VG. (2003). Antibiotic susceptibility of isolates of *Bacillus anthracis*, a bacterial pathogen	Focus Technologies, Herndon, VA, USA. mjones@focustechnologies.com *Bacillus anthracis* is a bacterial species that could be used in a bioterrorist attack. We tested a collection of isolates with a range of relevant antimicrobial compounds. All isolates tested were susceptible to ciprofloxacin and doxycycline. Penicillin and amoxicillin, with or without clavulanate, showed in vitro activity against all B. anthracis isolates. Ceftriaxone demonstrated lower-level in vitro activity compared to penicillin-related compounds against B. anthracis. In

with the potential to be used in biowarfare. Clin Microbiol Infect. 9(9):984-6.	vitro data from this study are in keeping with available guidelines.
Jones MN, Beedham RJ, Turnbull PCB et al: (1996). Efficacy of the UK human anthrax vaccine in guinea pigs against aerosolized spores of *Bacillus anthracis*. Salisbury Medical Bulletin, special supplement #87, June p 123	
Jones MN, Beedham RJ, Turnbull PCB et al: (1996). Antibiotic prophylaxis for inhalation anthrax.Salisbury Medical Bulletin, special supplement #87, June p 127	
Jones TR. (1937). Observations of a Stock Inspector in the Jerilderie Pastures Protection District, 1933-1936. Aust Vet J 64-72	'One outbreak involveing the loss of four beasts was reported. The outbreak was checked by inoculation. There seems to be a definite anthrax belt in the south east corner of the district …'
Jula GR M, Jabbari AR, Malek B. (2004). Isolation of anthrax spores from soil in endemic regions of Isfahan, Iran. Archives of Razi Institute. Razi Vaccine and Serum Research Institute, Teheran, Iran:. 58, 29-38.	To isolate and detect anthrax spores from soil in different regions of Isfahan, Iran, a total of 60 environmental specimens were collected during 2003. Bacterial endospores were extracted via flotation in distilled water and were cultured on blood agar and selective PLET media. *Bacillus anthracis* was identified using bacteriological and biological tests. Viable B. anthracis spores were isolated from 9 (15%) soil samples of the 60 collected specimens in which 6 (66%) of isolates were encapsulated. The isolated bacteria and their virulence were confirmed with polymerase chain reaction (PCR) using specific primers. Because of the existence of a highly virulent strain of B. anthracis in this region, a review on implementation of control programmes such as regular vaccination of all susceptible livestock and surveillance of the disease in animals and humans in such endemic areas are required.
Kadymov RA, Aliev RM. (1979). Simultaneous immunization of sheep (against anthrax, pasteurellosis and clostridial infections). Veterinariya, Moscow.. 5, 38-40.	20 of 45 sheep received separation injections of "STI" anthrax vaccine and a combined vaccine against Pasteurella multocida and Clostridium perfringens and C. septicum. The remaining sheep were vaccinated with single vaccines. Haematological changes were similar in the different groups. Serum samples were examined for neutralizing antibodies and agglutinins. Challenge infection with one of the four causal agents, carried out after 8 months, showed no difference in the survival rate between monovalent and polyvalent immunization. Inoculation of over 5000 sheep confirmed the safety of the combined method.
Kalns J, Morris J, Eggers J, Kiel J. (2002). Delayed treatment with doxycycline has limited effect on anthrax infection in BLK57/B6 mice. Biochemical and Biophysical Research Communications.. 297: 3, 506-509.	Blk57/B6 mice were infected with LD90 dose of Sterne strain anthrax spores subcutaneously and then treated with doxycycline. Doxycycline at a dose of 1.5 mg/kg, by intra-peritoneal injection, protected mice from death when given at the same time as spores. When doxycycline administration was delayed 4 h survival is 90%. Delay of 24 h increased survival time but had no impact on eventual mortality. When doxycycline was delayed 48 h, mortality and time to death were comparable to sham injection. Peritoneal macrophages harvested from Blk57/B6 mice were examined for response to anthrax lethal toxin and are shown to be deficient in their ability to produce TNF- alpha and have increased

	expression of IL-6 compared to RAW 264.7 murine macrophage cell line. These findings suggest that antibiotic therapy has limited effects following lethal anthrax spore challenge, even when the host is of a phenotype that does not produce TNF- alpha in response to anthrax lethal toxin exposure.
Karginov VA, Robinson TM, Riemenschneider J, Golding B, Kennedy M, Shiloach J, Alibek K (2004). Treatment of anthrax infection with combination of ciprofloxacin and antibodies to protective antigen of *Bacillus anthracis*. FEMS Immunology and Medical Microbiology 40: 71-74	
Kasianenko AM, Verner OM, Zaviriukha AM, Siniak KM. (1984). Mapping method in the epidemiological study of infections and infestations. II. Soil characteristics in localities permanently at risk for anthrax. Zhumal Mikrobiology Epidemiology Immunobiology January; 90-95.	[Russian]
Kaufman AF (1990). Observations on the occurrence of anthrax as related to soil type and rainfall. Proceedings of the international workshop on anthrax. Salisbury Medical Bulletin;68(suppl):16-17.	
Kaufmann AF (1993). Anthrax. *In* Current Veterinary Therapy 3 Food Animal Practice, edited by JL Howard, WB Saunders, Philadelphia,pp 565-567	
Kaufmann AF, Fox MD, Kolb RC (1973). Anthrax in Louisiana, 1971. An evaluation of the Sterne strain anthrax vaccine. Journal of the American Veterinary Medical Association 163: 442–445.	Retrospective survey with no concurrent on-farm controls (ie all animals vaccinated or not vaccinated). No information on vaccine dose (volume administered, spore concentration or route of administration) provided. The efficacy of the Sterne strain non-encapsulated avirulent spore vaccine was evaluated by surveying 73 farms, with an animal population of 4422 cattle, 29 horses and 20 pigs, during and after and epidemic of anthrax in Louisiana. Most of the deaths due to anthrax (97.4%) occurred either before or 1-7 days after vaccination. Although two doses of the vaccine were frequently given, a single dose provided equivalent protection. Studies on two premises that had vaccinated 15 months before the current outbreak suggested that active immunity may persist for more than one year.
Kaufmann AF, Meltzer MI, Schmid GP. (1997). The economic impact of a	Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA. Understanding and quantifying the impact of a bioterrorist attack are essential in developing public health preparedness

bioterrorist attack: are prevention and postattack intervention programs justifiable? Emerg Infect Dis. 3(2):83-94.	for such an attack. We constructed a model that compares the impact of three classic agents of biologic warfare (*Bacillus anthracis*, Brucella melitensis, and Francisella tularensis) when released as aerosols in the suburb of a major city. The model shows that the economic impact of a bioterrorist attack can range from an estimated $477.7 million per 100,000 persons exposed (brucellosis scenario) to $26.2 billion per 100,000 persons exposed (anthrax scenario). Rapid implementation of a postattack prophylaxis program is the single most important means of reducing these losses. By using an insurance analogy, our model provides economic justification for preparedness measures.
Keim P, Klevytska AM, Price LB, Schupp JM, Zinser G, Smith KL, Hugh-Jones ME, Okinaka R, Hill KK, Jackson PJ. (1999). Molecular diversity in *Bacillus anthracis*. J Appl Microbiol 87: 215-217	
Keim P, Price LB, Klevytska AM, Smith KL, Schupp JM, Okinaka R, Jackson PJ, Hugh-Jones ME. (2000). Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. Journal of Bacteriology 182:2928–2936.	Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011-56401 *Bacillus anthracis* is one of the most genetically homogeneous pathogens described, making strain discrimination particularly difficult. In this paper, we present a novel molecular typing system based on rapidly evolving variable-number tandem repeat (VNTR) loci. Multiple-locus VNTR analysis (MLVA) uses the combined power of multiple alleles at several marker loci. In our system, fluorescently labeled PCR primers are used to produce PCR amplification products from eight VNTR regions in the B. anthracis genome. These are detected and their sizes are determined using an ABI377 automated DNA sequencer. Five of these eight loci were discovered by sequence characterization of molecular markers (vrrC1, vrrC2, vrrB1, vrrB2, and CG3), two were discovered by searching complete plasmid nucleotide sequences (pXO1-aat and pXO2-at), and one was known previously (vrrA). MLVA characterization of 426 B. anthracis isolates identified 89 distinct genotypes. VNTR markers frequently identified multiple alleles (from two to nine), with Nei's diversity values between 0.3 and 0.8. Unweighted pair-group method arithmetic average cluster analysis identified six genetically distinct groups that appear to be derived from clones. Some of these clones show worldwide distribution, while others are restricted to particular geographic regions. Human commerce doubtlessly has contributed to the dispersal of particular clones in ancient and modern times. Includes 30 isolates and 3 genotypes from Australia (EMAI and others)
Keim P, Smith KL, Keys C, Takahashi H, Kurata T, Kaufmann A. (2001). Molecular Investigation of the Aum Shinrikyo Anthrax Release in Kameido, Japan. J Clin Microbiol 39: 4566-4567	In 1993, the Aum Shinrikyo cult aerosolized *Bacillus anthracis* spores over Kameido, Japan. Spore samples were obtained from the release site, cultured, and characterized by molecular genetic typing. The isolates were consistent with strain Sterne 34F2, which is used in Japan for animal prophylaxis against anthrax.
Kennedy D. (1979). A study of anthrax in grazing livestock. Thesis, Master of Veterinary Studies in Veterinary Preventive Medicine and Epidemiology. University of Melbourne.	
Kenney RT, Yu J, Guebre-Xabier M, Frech SA, Lambert A, Heller BA, Ellingsworth LR, Eyles JE, Williamson ED, Glenn GM.	IOMAI, Gaithersburg, Maryland 20878, USA. rkenney@iomai.com BACKGROUND: Transcutaneous immunization (TCI) is a needle-free technique that delivers antigens and adjuvants to potent epidermal immune cells. To address critical unmet needs in biodefense against anthrax, we have designed a

(2004). Induction of protective immunity against lethal anthrax challenge with a patch. J Infect Dis 190(4): 774-782	novel vaccine delivery system using a dry adhesive patch that simplifies administration and improves tolerability of a subunit anthrax vaccine. METHODS: Mice and rabbits were vaccinated with recombinant protective antigen of *Bacillus anthracis* and the heat-labile toxin of Escherichia coli. Serologic changes, levels of toxin-neutralizing antibodies (TNAs), and pulmonary and nodal responses were monitored in the mice. A lethal aerosolized B. anthracis challenge model was used in A/J mice, to demonstrate efficacy. RESULTS: The level of systemic immunity and protection induced by TCI was comparable to that induced by intramuscular vaccination, and peak immunity could be achieved with only 2 doses. The addition of adjuvant in the patch induced superior TNA levels, compared with injected vaccination. CONCLUSIONS: Anthrax vaccine patches stimulated robust and functional immune responses that protected against lethal challenge. Demonstration of responses in the lung suggests that a mechanism exists for protection against challenge with aerosolized anthrax spores. A formulated, pressure-sensitive, dry adhesive patch, which is stable and can be manufactured in large scale, elicited comparable immunoglobulin G and TNA responses, suggesting that an anthrax vaccine patch is feasible and should advance into clinical evaluation.
Keppie J, Smith H, Harris-Smith PW. (1955). The chemical basis of the virulence of *Bacillus anthracis*. III: The role of the terminal bacteraemia in death of guinea pigs from anthrax. Br J Exp Pathol;136:315-322.	
Khalifa HW, Zaki S. (1982). Comparative studies on the biochemical and immunogenic properties of three strains of *Bacillus anthracis*. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases.. 3: 4, 231-232.	
Kiel JL, Parker JE, Alls JL, Kalns J, Holwitt EA, Stribling LJV, Morales PJ, Bruno JG (2000). Rapid recovery and identification of anthrax bacteria from the environment. Ann NY Acad Sci 240-252	Liquid medium Culture conditions and sporulation
Kiel JL, Parker JE, Gifford H, Stribling LJV, Alls AJ, Meltz ML, McCreary RP, Holwitt EA. (2002). Basis for the extraordinary genetic stability of anthrax. Ann NY Acad Sci 969: 112-118	500 isolates from around the world represent one of the most genetically homogenous microbes. Environmental factors (related to nitration tolerance) acting as selective agents play the overwhelming role in maintaining genetic stability.
Kihira T, Sato J, Shibata T. (2004). Pharmacokinetic-pharmacodynamic analysis of fluoroquinolones against *Bacillus anthracis*. J Infect Chemother. 10(2):97-100.	Pharmaceuticals and Medical Devices Evaluation Center, National Institute of Health Sciences, Toranomon 33 Mori Building, 10F, 3-8-21 Toranomon, Minato-ku, Tokyo 105-8409, Japan. kihira@nihs.go.jp Based on the pharmacokinetic-pharmacodynamic (PK-PD) parameters of ciprofloxacin in rhesus monkeys, the efficacies of levofloxacin, sparfloxacin, norfloxacin, and tosufloxacin against anthrax in humans were examined. The optimal PK-PD parameter for the prophylaxis or treatment of infection with *Bacillus anthracis* is not clearly defined. To evaluate the efficacy of fluoroquinolones against anthrax, PK-PD parameters and the protein-binding effect of

	fluoroquinolones are used. B. anthracis is very susceptible to fluoroquinolones in vitro, and levofloxacin, sparfloxacin, and tosufloxacin may be as effective against anthrax as ciprofloxacin by PK-PD analysis. However, additional studies of the in vivo model are necessary to define more clearly efficacy against anthrax and the pharmacodynamic relationship of fluoroquinolones.
Kleine-Albers C, Bohm R. (1989). Detection of *Bacillus anthracis* protective antigens by enzyme-immunoassay using polyclonal and monoclonal antibodies. Journal of Veterinary Medicine, B. 36: 3, 226-230.	An enzyme-immunoassay (EIA) for the detection of *Bacillus anthracis*-protective antigen (PA) within one hour was developed. If the rabbit antiserum was used, 15 ng PA/ml could be detected and with monoclonal antibody, the detection limit was 60 ng PA/ml. With respect to the higher specificity and with regard to the aspects of animal care, it is recommended that monoclonal antibodies should be used in the test instead of the polyclonal antiserum.
Kleine-Albers C. (1988). Demonstration of *Bacillus anthracis* protective antigen by enzyme immunoassay using polyclonal and monoclonal antibodies. Justus-Liebig-Universitat Giessen, German Federal Republic:. 140.	
Klemm DM, Klemm WR (1959). A history of anthrax. J Am Vet Med Assoc 135: 458-462	
Klinman DM, Xie H, Little SF, Currie D, Ivins BE. (2004). CpG oligonucleotides improve the protective immune response induced by the anthrax vaccination of rhesus macaques. Vaccine. 22(21-22):2881-6.	Section of Retroviral Immunology, Center for Biologics Evaluation and Research, Food and Drug Administration, Bldg. 29A, Rm. 3 D 10, Bethesda, MD 20892, USA. klinman@cber.fda.gov Synthetic oligodeoxynucleotides (ODN) containing unmethylated CpG motifs act as immune adjuvants, improving the immune response elicited by co-administered vaccines. Combining CpG ODN with anthrax vaccine adsorbed (AVA, the licensed human vaccine) increased the speed, magnitude and avidity of the resultant anti-anthrax response. The protective activity of these Abs was established by passive transfer to anthrax-challenged mice. The ability of CpG ODN to accelerate and magnify the immune response to AVA suggests this strategy may contribute to the development of prophylactic and therapeutic vaccines against biothreat pathogens.
Knudson GB (1986). Treatment of anthrax in man: history and current concepts. Mil Med 151:71-77	Penicillin, chlortetracycline, sulfonamides etc Immunity to anthrax is not an all-or-none type of resistance but rather a graded response. The Vollum strain can overwhelm immunized animals if dose large enough.
Knudson GB, Little SF, Ivins BE. (1988). Immunization against anthrax with a transposon Tn916-generated aro mutant of *Bacillus anthracis*. Abstracts of the Annual Meeting of the American Society for Microbiology.. 88: 105.	
Kobiler D, Gozes Y, Rosenberg H, Marcus D, Reuveny S, Altboum Z. (2002). Efficiency of protection of guinea pigs	The efficacy of passive immunization as a postexposure prophylactic measure for treatment of guineapigs intranasally infected with *Bacillus anthracis* spores was evaluated. Antisera directed either against the lethal toxin components (PA or LF) or against a toxinogenic strain (Sterne) were used for this evaluation. All antisera exhibited high enzyme-linked

against infection with *Bacillus anthracis* spores by passive immunization. Infection and Immunity. . 70: 2, 544-550.	immunosorbent assay titres against the corresponding antigens, high titres of neutralization of cytotoxicity activity in an in vitro mouse macrophages cell line (J774A.1), as well as in vivo neutralization of toxicity when administered either directly to Fisher rats prior to challenge with the lethal toxin or after incubation with the lethal toxin. In these tests, anti-LF antiserum exhibited the highest neutralization efficiency, followed by anti-Sterne and anti-PA. The time dependence and antibody dose necessary for conferring postexposure protection by the various antibodies of guineapigs infected with 25 50% lethal doses of Vollum spores was examined. Rabbit anti-PA serum was found to be the most effective. Intraperitoneal injections of anti-PA serum given 24 h postinfection protected 90% of the infected animals, whereas anti-Sterne and anti-LF were less effective. These results further emphasizes the importance of anti-PA antibodies in conferring protection against B. anthracis infection and demonstrated the ability of such antibodies to be effectively applied as an efficient postexposure treatment against anthrax disease.
Kobuch WE, Davis J, Fleischer K, Isaacson M, Turnbull PCB. (1990). A clinical and epidemiological study of 621 patients with anthrax in western Zimbabwe. Proceedings of the international workshop on anthrax. Salisbury Medical Bulletin;68(suppl):34-38.	
Koch R (1876). [The etiology of anthrax, based on the life history of *Bacillus anthracis*.] Beitrage zur Biologie der Pflanzen 2: 277-310	
Kogotova OI, Buravtseva NP. (1993). The nonspecific resistance indices of different species of laboratory animals immunized with STI anthrax vaccine. Zh Mikrobiol Epidemiol Immunobiol. (2): 89-92.	A complex comparative study of the characteristics of nonspecific resistance in different species of laboratory animals immunized with vaccine STI against anthrax has revealed the existence of marked interspecific differences between noninbred white mice, guinea pigs, rabbits and noninbred white rats in such characteristics as phagocytic activity, oxygen-dependent function of polymorphonuclear blood leukocytes, serum beta-lysin and lysozyme.
Kolavic S, Kimura A, Simons S, Slutsker L, Barth S, Haley C. (1997). An outbreak of Shigella dysenteriae Type 2 among laboratory workers due to intentional contamination. J. Am. Med. Assoc. 278, 396– 398.	[In 1996 a hospital worker in Texas was suspected of using stock cultures of Shigella to contaminate muffins and doughnuts left in the staff room.]
Kolesov SG, Rudenko LP, Romanov GI, Solomatin VI. (1976). Duration of immunity in sheep vaccinated against anthrax. Veterinariya, Moscow.. 9, 33-34.	Two Russian vaccines were given to 83 sheep in doses of 12, 16, 18 or 24 million spores. After 12, 19 and 24 months the vaccinated sheep were challenged with virulent *Bacillus anthracis*. Both vaccines were highly immunogenic and produced a stable, prolonged immunity lasting at least 2 years. There was no significant difference in the immunity produced by the different vaccination doses.
Komiyama T, Swanson FA, Fuller RS. (2005). Protection from Anthrax Toxin-Mediated Killing of Macrophages by the	Departments of Biological Chemistry,1 Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan 481092 bfuller@umich.edu Cell surface proteolytic processing of anthrax protective antigen by furin or other furin-related proteases is required for

Combined Effects of Furin Inhibitors and Chloroquine. Antimicrobial Agents and Chemotherapy 49(9): 3875-3882	its oligomerization, endocytosis, and function as a translocon for anthrax lethal and edema factors. Countering toxin lethality is essential to developing effective chemotherapies for anthrax infections that have proceeded beyond the stage at which antibiotics are effective. The primary target for toxin is the macrophage, which can be killed by lethal factor via both necrotic and apoptotic pathways. Here we show that three high-affinity inhibitors of furin efficiently blocked killing of murine J774A.1 macrophages by recombinant protective antigen plus lethal factor: RRD-eglin and RRDG-eglin, developed by engineering the protein protease inhibitor eglin c, and the peptide boronic acid inhibitor acetyl-Arg-Glu-Lys-boroArg pinanediol. Inhibition of killing was dose dependent and correlated with prevention of protective antigen processing. Previous studies have shown that weak bases, such as chloroquine, which neutralize acidic compartments, also interfere with toxin-dependent killing. Here we show that combining furin inhibitors and chloroquine strongly augments the inhibition of toxin-dependent killing, suggesting that combined use of antifurin drugs and chloroquine might provide enhanced therapeutic benefits. Reversible furin inhibitors protected against anthrax toxin killing for at least 5 h, but by 8 h, toxin-dependent killing resumed even though furin inhibitors were still active. An irreversible chloromethylketone inhibitor did not exhibit this loss of protection.
Koprowski H. (2002). Old and new prescriptions for infectious diseases and the newest recipes for biomedical products in plants. Arch Immunol Ther Exp (Warsz). 50(6): 365-9.	Biotechnology Foundation Laboratories at Thomas Jefferson University, Philadelphia, PA, USA. The three antiviral vaccines discovered in the 18th century (smallpox), 19th century (rabies), and 20th century (polio) share a common feature: none would ever be licensed today for human vaccination. Yet Jenner's smallpox vaccine led to the eradication of smallpox, Pasteur's rabies vaccine represented the first successful post-exposure treatment of people bitten by rabid animals, and polio vaccine administered since its discovery in 1950 is leading to the eradication of polio (in the years 2004-2005) from the earth. However, in the case of rabies, efforts at complete eradication are unrealistic, despite the availability of a very effective vaccine, since rabies, unlike smallpox and polio, is not limited to humans and can infect all domestic and wild mammalian species. Rabies is probably the oldest known infectious disease, yet knowledge of the virus and the disease is far from complete. For instance, the appearance of 24 cases of "cryptic" rabies in the USA, i.e. cases not associated with any bite or scratch, with an incubation period in humans extending 6-8 years, is a puzzling phenomenon that cannot be readily explained. On the other hand, rabies is one of the few strictly neuronal infections and, as such, is an excellent model for the study of neurotropic virus distribution in the brain. Apoptosis induced by a rabies strain expressing high levels of glycoprotein spreads much more slowly through brain tissue than that induced by strains producing lower glycoprotein levels. Attenuated rabies virus constructed to express twice the normal glycoprotein levels is also an excellent antigen for induction of immune responses in the host. Foreign antigens using this vector may also produce highly immunogenic vaccines. Global approach to immunization. Those monitoring the spread of AIDS in many parts of the world know that cost of treatment is one of the major problems in combating the disease. Vaccines against HIV face the same problem. In general, the price of vaccines and sera is exorbitant for the afflicted population in developing countries. In addition, the dearth of syringes, the unavailability of nurses and doctors to administer multiple vaccine injections, and other factors in these countries require a drastic change in current vaccine production approaches. About 12 years ago, plants became vehicles to produce biomedical reagents. Plants can be exposed directly to a construct containing a foreign gene and Agrobacterium to create a transgenic plant that, over several generations, produces the desired product. Alternatively, plants infected with a plant virus (e.g. alfalfa mosaic virus) fused with a foreign gene can propagate the foreign antigen as the virus multiplies. Extraction of the plant virus followed by purification provides the desired biomedical product. Our use of either of these systems has led to the creation of plants producing vaccines, sera, hormones, and other biological reagents. In two clinical trials at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences in Poznan (Poland), volunteers who ingested lettuce expressing hepatitis B vaccine showed hepatitis B antibodies in their sera. In

	another trial carried out at the Biotechnology Foundation Laboratories in Philadelphia (USA), volunteers ingesting a spinach-rabies vaccine showed an immunological priming effect, since only one injection of commercially available rabies vaccine significantly raised the level of rabies-specific antibodies. Vaccines against HIV gp120 and Tat have been produced in spinach, and a construct of gp120 with the CD4 receptor is now being adapted to this plant. Two types of antibodies against rabies and against colorectal cancer are being produced in tobacco and in lettuce. The suboptimal quality of the currently available anthrax vaccine prompted our efforts to produce the anthrax protective antigen (PA) in tobacco and lettuce. Quite clearly, plants will play a prominent role in producing a variety of biomedical reagents in the future.
Koprowski H. (2005). Vaccines and sera through plant biotechnology. (Plant-derived vaccines and antibodies: potential and limitations.) Vaccine.. 23: 15, 1757-1763.	A brief history of the production of plant-derived vaccines and sera is presented together with new advances in this field, particularly in the production of plant-derived vaccines against respiratory syncytial virus infections, hepatitis B, human immunodeficiency virus, rabies, anthrax, diphtheria, severe acute respiratory syndrome, and smallpox. The production of vaccines using virus vectors is compared with that with transgenic plants and the efficacy of vaccines produced via plant biotechnology is illustrated.
Korotich AS, Pogrebnyak LI. (1976). Anthrax. Sibirskaya yazva.. Izdatel'stvo "Urozhai"., Kiev, Ukrainskaya SSR, USSR:. 160.	This monograph gives a brief historical review, and an account of the spread of the disease, especially in the USSR. The morphology of B. anthracis, its sporulation, encapsulation and cultural characteristics are described with line drawings. Epidemiology, clinical signs, pathogenesis, lesions and diagnosis of anthrax are discussed. The use of ethylene oxide/methyl bromide mixture to disinfect the soil is described; also the use of biological methods, with Escherichia coli, Chlorella or Scenedesmus species, or Actinomycetales. Prophylactic and control measures are also described.
Kosal ME, Anderson DE (2004). An unaddressed issue of agricultural terrorism: a case study on feed security. J Anim Sci 82: 3394-3400	Accidental: salinomycin in feed for alpacas Definition: Agriterrorism: the malicious use of plant or animal pathogens to cause devastating disease in the agricultural sector (FEMA 2002 www.fema.gov/pdf/onp/toolkit_app_e.pdf) Discussion: Chemical: chlordane intentionally added to rendering plant material that then added to animal feed; OP contamintation of silo contents of animal feed; insecticide contaminaton of feed in Nebraska feedlot; PBB episode in Michigan; dioxin contamination in Belgium; plant toxins contaminating feed. Biological: anthrax infected cattle cakes; FMD, African swine fever, rinderpest, use of 'rabbit smoothies' – homogenized tissues of rabbits with calicivirus (NZ); BSE.
Kraus H, Weber A, Appel M, Enders B, Isenberg HD, Shiefer HG, Slenczka W, von Graevenitz A, Zahner H. (2003). Zoonoses, 3rd edition ASM Press, Washington DC, Anthrax pp 173-176	
Krishna Rao NS, Mohiyudeen S. (1958). Tabanus flies as transmitters of anthrax: a field experience. Indian Veterinary Journal;35:348-353.	
Kuoni E, Zindel W. (1986). Anthrax in 1985 in Graubunden Canton, Switzerland. Schweizer Archiv fur Tierheilkunde.. 128:	Single cases of anthrax in cattle occurred in the Canton in 1960, 1966, 1967, 1969 and 1972. In 1985 the disease affected 11 cattle and 2 goats during July, attributed to the bleeding-out of a moribund cow (later found to have anthrax) on a pasture. 3821 cattle in the area were vaccinated.

5, 261-267.	
Lacy DB, Wigelsworth DJ, Melnyk RA, Harrison SC, Collier RJ. (2004). Structure of heptameric protective antigen bound to an anthrax toxin receptor: A role for receptor in pH-dependent pore formation PNAS 101: 13147-13151	
Laforce FM (1978). Woolsorters' disease in England. Bulletin of the New York Academy of Medicine 54:956–963	
Lamarque D, Haessler C, Champion R, Granga D, Bendina D, Steinmetz P, Guelina A, Maurice Y. (1989). Anthrax in Chad, a continuing zoonosis. Medecine Tropicale.. 49: 3, 245-251.	Anthrax is endemic in the Sahelian regions of Chad, appearing each year in the rainy season, but in 1988, a year of exceptionally heavy rainfall, an epidemic in the Chari Baguirmi Prefecture from August to December affected over half the population of donkeys and horses. Of the 7453 donkeys 52% were affected and 39% died, and of the 1195 horses 38% were affected and 21% died. Of 716 human cases reported, mainly from eating contaminated meat, 88 died. Cattle were also affected, and two cases in pigs were seen. Compulsory vaccination of 300 000 cattle was carried out. Two strains of *Bacillus anthracis* were isolated from cattle and pigs, but not from donkeys, horses or human cases, nor from tabanid flies which were exceptionally numerous and which were considered to have spread the disease among donkeys and horses, and also to man. Because of the impossibility of destroying the numerous scattered carcasses, annual flare-ups of the disease are predicted.
Lamb A. (2001). Biological weapons: the facts not the fiction. Clin. Med. 1 (6), 502– 504.	[There is also an indication that Germany had planned to drop typhus and plague infected rats over England during the second World War (Lamb, 2001). With the knowledge that German rockets were loaded with biological agents, Winston Curchill had ordered preparations to retaliate by dropping anthrax-contaminated cattle cakes in German countryside to affect the beef supplies.]
Launder WH (1932). Longevity of anthrax spores. Lancet 220(5691): 707	Spores on agar culture survived 18.5 years. Storage conditions not described.
Lawrence D, Heitefuss S, Seifert HSH. (1991) Differentiation of *Bacillus anthracis* from Bacillus cereus by gas chromatographic whole-cell fatty acid analysis. Journal of Clinical Microbiology.. 29: 7, 1508-1512.	Three strains of B. anthracis and 7 strains of B. cereus were grown on complex medium and on synthetic medium. Gas chromatographic analysis of whole-cell fatty acids of strains grown on complex medium gave nearly identical fatty acid patterns. Fatty acid patterns of strains grown on synthetic medium showed a high content of branched-chain fatty acids. Significant differences between the fatty acid patterns of the 2 species were found. Odd iso/anteiso fatty acid ratios were about equal in B. anthracis strains, whereas in B. cereus strains the fractions of iso acids were at least twice as high as the fractions of anteiso acids. This method is used in a diagnostic laboratory to help differentiate between these species.
Lawrence JA, Foggin CM, Norval RA. (1980). The effects of war on the control of diseases of livestock in Rhodesia (Zimbabwe). Vet Rec;107:82-85.	
Lee JiYoun; Yoo HanSang; Kim JongYeom.	[Korean] A fast and specific diagnostic technique using a polymerase chain reaction (PCR) was developed to detect B.

(1998). Development of a polymerase chain reaction to detect *Bacillus anthracis* in soil and mice. Korean Journal of Veterinary Research.. 38: 3, 574-580.	anthracis in soil and experimentally infected mice. Virulence genes of B. anthracis were amplified from experimentally inoculated soil and mice. Up to 4.2x10 organisms per gram of soil could be detected using the PCR technique. It is suggested that this PCR method could be used effectively to detect B. anthracis in soil and infected animals and may be instrumental in disease prevention and control.
Lee LA, Kidd ARM, Sterne M. (1961). Antibiotic treatment and its effect on anthrax immunisation. Vet Rec 73: 1426	Inoculated cow died of anthrax 1 month after vaccination. Cow had had metritis and had been treated with penicillin and streptomycin within 3 days of vaccination. Cautioned that antibiotics are also commonly used in feedstuffs.
Lee LV, Brower KE, Liang F-S, Shi J, Wu D, Sucheck SJ, Vogt PK, Wong C-H (2004). Inhibition of the proteolytic activity of anthrax lethal factor by aminoglycosides. J Am Chem Soc 126: 4774-4775	Neomycin B
Lee MA, Brightwell G, Leslie D, Bird H, Hamilton A. (1999). Fluorescent detection techniques for real-time multiplex strand specific detection of *Bacillus anthracis* using rapid PCR. J Appl Microbiol 87: 218-223	
Leitenberg M. (2001). Biological weapons in twentieth century: a review and analysis. Crit. Rev. Microbiol. 27 (4), 267– 320.	[During WW I Germany is said to have used anthrax and glanders in an effort to destroy animals used during the war.]
Lensing HH, Oei HL. (1985) Investigations on the sporicidal and fungicidal activity of disinfectants. Zentralbl Bakteriol Mikrobiol Hyg [B]. 181(6):487-95.	The sporicidal and fungicidal activity of disinfectants was studied in a suspension test. Glutaraldehyde 4%, sodium-dichloroisocyanurate-dihydrate (2400 ppm active chlorine) and peracetic acid 0.25% demonstrated after 30 min of exposure at 20 degrees C in the presence of 4% horse serum a clear activity against spores of Bacillus cereus. Under the same conditions formaldehyde 4% and glutaraldehyde 2% were also found to be sporicidal, but only after a longer time of exposure. Spores of *Bacillus anthracis* and B. cereus appeared to be comparably resistant against the investigated disinfectants, whereas conidiospores of Aspergillus fumigatus and Aspergillus niger were less resistant. Of the micro-organisms tested Candida albicans proved to be slightest resistant, while spores of Bacillus subtilis were found the most resistant.
Leppla SH (1982). Anthrax toxin edema factor: a bacterial adenylate cyclase that increases cyclic AMP concentrations of eukaryotic cells. Proc Natl Acad Sci USA 79: 3162–6.	[During et al 2001, 6]
Leppla SH, Arora N, Varughese M.(1999). Anthrax toxin fusion proteins for intracellular delivery of macromolecules. J Appl Microbiol 87: 284	
Leppla SH, Robbins JB, Schneerson R, Shiloach J. (2002). Development of an	

improved vaccine for anthrax. Journal of Clinical Investigation 110: 2, 141-144	
Lesniak OT, Saltykov RA. (1970). A comparative evaluation of the immunogenicity of anthrax vaccine strains. Zh Mikrobiol Epidemiol Immunobiol. 47(8):32-5.	
Levi K, Higham JL, Coates D, Hamlyn PF. (2003). Molecular detection of anthrax spores on animal fibres. Letters in Applied Microbiology. . 36: 6, 418-422.	Aims: To develop a rapid, specific and sensitive diagnostic test for the detection of the spores of *Bacillus anthracis* on commercial samples of animal fibres (e.g. wool and cashmere). Methods and Results: Extraction of DNA from spores using a mechanical disruption method based on bead beating was evaluated but subsequently abandoned as it compromised the sensitivity of the overall protocol. A multiplex PCR and two nested amplification reactions designed for B. anthracis were developed during this study. Conclusions: A simple selective incubation step in combination with multiplex PCR was found to be more effective than generic DNA extraction coupled to a sensitive nested amplification reaction. Significance and Impact of the Study: The rapid diagnostic test could be applied to the analysis of commercial fibre samples for the detection of anthrax as required by health and safety legislation resulting in considerable savings in time and expense.
Liang X, Yu D. (1999). Identification of *Bacillus anthracis* strains in China. J Appl Microbiol 87: 200-203	
Liddington R, Pannifer A, Hanna P, Leppla S, Collier RJ. (1999). Crystallographic studies of the anthrax lethal toxin. J Appl Microbiol 87: 282	
Lim NK, Kim JH, Oh MS, Lee S, Kim S-Y, Kim K-S, Kang H, Hong H, Inn K-S (2005). An Anthrax Lethal Factor-Neutralizing Monoclonal Antibody Protects Rats before and after Challenge with Anthrax Toxin. Infect Immun 73: 6547-6551.	Aprogen, #311 BVC, Korea Research Institute of Bioscience and Biotechnology, 52 Eoeun-dong, Yuseong-gu, Daejon 305-333, South Korea. iks0808@aprogen.co.kr Lethal factor (LF) is a component of anthrax lethal toxin (LeTx). We generated anti-LF murine monoclonal antibodies (MAbs) that show LeTx-neutralizing activity in vitro and in vivo. Anti-LF MAbs were generated by immunization with recombinant LF, and the MAbs showing LeTx-neutralizing activity in vitro were selected. Two MAbs with the highest affinities, 5B13B1 (dissociation constant [Kd], 2.62 nM) and 3C16C3 (Kd, 8.18 nM), were shown to recognize the same or closely overlapping epitopes on domain III of LF. The 50% inhibitory concentration of 5B13B1 (0.21 μg/ml) was approximately one-third that of 3C16C3 (0.63 μg/ml) in the in vitro LeTx-neutralization assay. The 5B13B1 antibody, which had the highest neutralizing activity, provided perfect protection against LeTx challenge in an in vivo LeTx neutralization assay using Fisher 344 rats. In addition, the antibody showed pre- and postexposure prophylactic effects in the animal experiments. This is the first report that an MAb binding to domain III of LF has neutralizing activity against LeTx. The 5B13B1 antibody may be useful in prophylaxis against anthrax poisoning.
Lincoln RE, Walker JS, Klein F, Rosenwald AJ, Jones WI. (1967). Value of field data for extrapolation in anthrax. Federation Proceedings 26: 1558-1562.	Discussion of data leading to the hypothesis that species naturally fall into two classes: 1. those resistant to establishment of anthrax but once established are susceptible to the toxin. 2. species which are susceptible to the establishment of disease but resistant to the toxin.

Lincoln RE, Walker JS, Klein P, Haines BW (1964). Anthrax. Adv Vet Sci 9: 327-362	Signs, pathogenesis, vaccination, diagnosis, treatment. Penicillin, streptomycin, oxytetracycline, chlortetracycline.
Lindeque PM and Turnbull PCB (1994). Ecology and epidemiology of anthrax in the Etosha National Park, Namibia. Onderstepoort Journal of Veterinary Research 61:71–83	Camden
Lindley EP. (1965). Anthrax – the carrier state in goats. Br Vet J 111: 215	
Lindley WH (1963). Anthrax vaccination. J Am Vet Med Assoc 142: 621-623	
Little SF, Ivins BE, Fellows PF, Friedlander AM. (1997). Passive protection by polyclonal antibodies against *Bacillus anthracis* infection in guinea pigs. Infect Immun. 65(12):5171-5.	Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21702-5011, USA. The protective effects of polyclonal antisera produced by injecting guinea pigs with protective antigen (PA), the chemical anthrax vaccine AVA, or Sterne spore vaccine, as well as those of toxin-neutralizing monoclonal antibodies (MAbs) produced against PA, lethal factor, and edema factor, were examined in animals infected with *Bacillus anthracis* spores. Only the anti-PA polyclonal serum significantly protected the guinea pigs from death, with 67% of infected animals surviving. Although none of the MAbs was protective, one PA MAb caused a significant delay in time to death. Our findings demonstrate that antibodies produced against only PA can provide passive protection against anthrax infection in guinea pigs.
Little SF, Ivins BE, Fellows PF, Pitt ML, Norris SL, Andrews GP. (2004). Defining a serological correlate of protection in rabbits for a recombinant anthrax vaccine. Vaccine. 22(3-4):422-30.	Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702-5011, USA. stephen.little@amedd.army.mil In these studies, a serological correlate of protection against anthrax was identified in New Zealand white (NZW) rabbits that had been given one or two injections of various amounts of recombinant protective antigen (rPA) combined with aluminum hydroxide adjuvant (Alhydrogel). Rabbits were subsequently challenged by the aerosol route with spores of the Ames isolate of *Bacillus anthracis*. Results suggested that the antibody response, as determined by the quantitative anti-rPA IgG ELISA and toxin neutralizing antibody (TNA) assay, were significant predictors ($P<0.0015$) of protection against a B. anthracis aerosol spore challenge in rabbits.
Little SF, Ivins BE. (1999). Molecular pathogenesis of *Bacillus anthracis* infection. Microbes and Infection.. 1: 2, 131-139.	
Little SF, Knudson GB. (1986). Comparative efficacy of *Bacillus anthracis* live spore vaccine and protective antigen vaccine against anthrax in the guinea pig. Infection and Immunity.. 52: 2, 509-512.	Several strains of B. anthracis have been reported previously to cause fatal infection in immunized guinea pigs. In this study, guinea pigs were immunized with either a protective antigen vaccine or a live Sterne strain spore vaccine, then challenged with virulent B. anthracis strains isolated from various host species from the United States and foreign sources. Confirmation of previously reported studies (which used only protective antigen vaccines) was made with the identification of 9 of the 27 challenge isolates as being vaccine resistant. However, guinea pigs immunized with the live Sterne strain spore vaccine were fully protected against these nine isolates. In experiments designed to determine the

	basis of vaccine resistance, guinea pigs which were immunized with individual toxin components and which demonstrated ELISA antibody titres comparable to those induced by Sterne strain vaccine were not protected when challenged with a vaccine-resistant isolate. Antibodies to toxin components may not be sufficient to provide protection against all strains of B. anthracis, and other antigens may play a role in active immunity. The efficacy of anthrax vaccines must be tested by using vaccine-resistant isolates if protection against all possible challenge strains is to be assured.
Little SF, Webster WM, Ivins BE, Fellows PF, Norris SL, Andrews GP. (2004). Development of an in vitro-based potency assay for anthrax vaccine. Vaccine. 22(21-22):2843-52.	United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, MD 21702-5033, USA. stephen.little@amedd.army.mil The potency assay currently used to evaluate consistency of manufacture for the anthrax vaccine is contingent upon meeting specified parameters after statistical analysis of the percent survival and time to death of vaccinated guinea pigs after challenge with spores of a virulent strain of *Bacillus anthracis*. During the development of a new anthrax vaccine based upon recombinant protective antigen (rPA) adsorbed to aluminum hydroxide gel (Alhydrogel), we found that the serological response of female A/J mice, as measured by a quantitative anti-rPA IgG ELISA, may be an effective method to monitor a manufacturer's consistency for rPA-based vaccines. An advantage of the proposed in vitro-based potency assay is that it will not need stringent biosafety containment measures as required by the current guinea pig potency assay.
Little SF, Webster WM, Norris SL, Andrews GP. (2004). Evaluation of an anti-rPA IgG ELISA for measuring the antibody response in mice. Biologicals. 32(2):62-9.	United States Army Medical Research Institute of Infectious Disease, Bacteriology Division, 1425 Porter Street, Fort Detrick, Frederick, MD 21702-5011, USA. stephen.little@amedd.army.mil A recombinant protective antigen (rPA)-based enzyme-linked immunosorbent assay (ELISA) was developed to measure the serological response of female A/J mice after inoculation with the new rPA-based anthrax vaccine. Several fundamental parameters of the ELISA were evaluated: specificity, precision, accuracy, linearity, and stability. Experimental results suggested that the quantitative anti-rPA IgG ELISA could be used to measure antibody levels in female A/J mice and may be useful as a potency assay to monitor consistency of manufacture of a rPA-based vaccine for planned clinical trials.
Logan NA, Carman JA, Melling J, Berkeley RCW. (1985). Identification of *Bacillus anthracis* by API tests. Journal of Medical Microbiology.. 20: 1, 75-85.	API and morphological tests were examined for ability to distinguish between 37 B. anthracis strains (virulent and avirulent) and 194 strains of closely related species (B. cereus, B. mycoides and B. thuringiensis). 34 strains of B. anthracis and 4 of B. cereus were tested by several other methods including capsule formation, growth on a selective medium, and phage sensitivity. Virulent strains of B. anthracis were easily separated from the closely related Bacillus species by most of the tests, but slightly virulent and avirulent strains of B. anthracis were separable from the closely related species only by API and phage-sensitivity tests.
Loir MA, Stanely E (1891a). Susceptibility of kangaroo to anthrax.Agric Gazette NSW 2: 206-207	Studies at Rodd Island Laboratory: full grown kangaroo died 28 hours after inoculation.
Loir MA, Stanley E. (1891b). Susceptibility of kangaroo to anthrax. Agric Gazette NSW 2: 456-457	Kangaroo rat died 35 hours after inoculation with a culture of B anthracis in beef tea. A native bear (koala?) died 42 hours after inoculation. A native cat died after being inoculated but a second native cat survived ingestion of a culture.
Loir MA. (1891). Notes on the large death rate among Australian sheep, in country infected with Cumberland Disease, or splenic fever. J Roy Soc NSW 25: 46-52.	200,000 sheep die each year. “At the present time, moreover, it is not only of the gravest importance to arrest the effects of contagious diseases in stock in order that the number of flocks and herds and the pastoral wealth of the country may be increased, but more especially, in view of the great impetus recently given to the meat export trade. Is it not, therefore, to the best interests of the Colonies to adopt every possible precaution to prevent European Bacteriologists from finding in Australian meat,

	microbes or remains of microbes in large quantities, a discovery to which the utmost publicity would be given by those interested in stopping the importation of foreign meat?" "Professor Anderson Stuart – There are always persons who would delight to find microbes, and English Agricultural paperw would use the fact with advantage to ring the death-knell of Australian meat in England for some time to come."
Long GW, O'Brien T. (1999). Antibody-based systems for the detection of *Bacillus anthracis* in environmental samples. J Appl Microbiol 87: 214	
Longchamp P, Leighton T. (1999). Molecular recognition specificity of *Bacillus anthracis* spore antibodies. J Appl Microbiol 87: 246-249	
Lovett J. (1997). Anthrax an elusive survivor. Search 28: 101	[Muller et al 2004: "… beef exports to Asia were at risk and Indonesia imposed a brief ban on beef and live cattle imports."]
Lucchesi PE, Gildersleeve N (1941). Treatment of anthrax. JAMA 116: 1506-1508	85
Lusis P, Smart N. (1996). Bovine anthrax in eastern Ontario. Canadian Veterinary Journal.. 37: 12, 747.	In July 1996, a 3-year-old Holstein cow was presented to the Kemptville Regional Veterinary Laboratory, Ontario, Canada for PM examination. Anthrax was diagnosed following culture of *Bacillus anthracis* from the spleen. It was noted that blood exudation from body orifices was slight and that capsules typical of B. anthracis were not observed on many bacilli stained on impression smears. It is concluded that veterinarians in Ontario should consider anthrax in differential diagnosis of cattle that die suddenly and that if anthrax is suspected before PM examination, blood smears from peripheral vessels of the unopened carcass should be examined for B. anthracis.
Lyon DG. (1973). An outbreak of anthrax at the Chester Zoological Gardens. Veterinary Record.. 92: 13, 334-337.	The paper records a second outbreak of anthrax at the Chester Zoological Gardens and describes the sequence of events, clinical signs in affected animals and methods of treatment and prophylaxis used. The post-mortem findings in one specimen are described. Public health aspects are mentioned because of the large number of persons involved in the distribution of unsterilised meat to carnivores on exhibition. It is interesting to note the varying degrees of susceptibility in the carnivores. Assuming that they all received infected meat, it seems that the small mammals were highly susceptible and that Felidae, while exhibiting some morbidity, were less susceptible. The Canidae and carnivorous birds appeared to be relatively resistant as none was affected.
M'Fadyean J (1909). Anthrax bacilli in milk. Journal of Comparative Pathology 22:148–149.	"…I have not been able to find any recorded instance in which the presence of the bacilli has been proved in the milk taken from the live subject." "… it is also probable that the milk invasion does not occur until the bacilli have begun to multiply in the circulating blood, and it is well known that that is an event which usually precedes death by an hour or two, or even less." "… the secretion of milk is practically suppressed at this stage of the illness." "It is only necessary … to withhold the milk of every cow that has a temperature above normal or which presents any other symptom of anthrax infection."

MacDiarmid SC, Thompson EJ (1997). The potential risks to animal health from imported sheep and goat meat. Rev Sci Tech OIE 16: 45-56	
MacDonald DW, Rawluk SR, Gannon WPJ. (1992). Anthrax in cattle. Canadian Veterinary Journal.. 33: 2, 135.	Anthrax was diagnosed in 4 herds of cattle in west and north central Alberta in 1991. Mortality in the 4 herds was 7 of 100, 7 of 102, 5 of 145 cows and 8 of 400 cattle, respectively. All of the deaths due to anthrax in the first 3 herds occurred within the last 2 weeks of August and the first 2 weeks of September. Losses in the 4th herd began in September and continued until late October. A total of 1050 contact animals were vaccinated with a live vaccine (Thraxol-2). The outbreak was preceded by an outbreak of anthrax in bison in Wood Buffalo National Park, but a relationship between the outbreaks could not be confirmed.
Macher A (2002). Industry-Related Outbreak of Human Anthrax, Massachusetts, 1868. Emerg Infect Dis 8: 1182	
MacVean DW, Espe BH. (1975). Occurrence of anthrax in Oklahoma in 1974. Oklahoma Veterinarian.. 27: 1, 18-21.	In July 1974, a small outbreak of anthrax occurred in cattle near Hydro, Oklahoma. Although laboratory and epidemiological evidence implicated anthrax as the cause of death or illness in a dozen or fewer animals in the herd, it appears to have been the largest episode of anthrax in Oklahoma since the major outbreak of 1957. A search of the pathology records for the past 16 years at the College of Veterinary Medicine, Oklahoma State University, revealed single cases of laboratory-confirmed bovine *Bacillus anthracis* infection in 1964 and 1965 in Craig County. Other sporadic incidents of anthrax have no doubt occurred in Oklahoma since 1957, but records were unavailable or lacking laboratory confirmation of the diagnosis.
Maglione E, Ginanni C, Cavallero G. (1971). Frequency and spread of anthrax in domestic animals in Italy and particularly in Piedmont. Annali della Facolta di Medicina Veterinaria di Torino 18: 339-351	[Italian] An epidemiological study of antrax in Italy is presented. Between 1953 and 1968 incidence of outbreaks of anthrax has decreased steadily from 800 to 147 cases per year and occurs predominantly in cattle. The disease is commoner in south central parts of the country, in which regions it is considered to be mainly soil-borne. In other parts it is more commonly associated with the hides and skins industry. Compulsory anti-anthrax vaccination is applied in certain provinces and vaccination to control outbreaks can be imposed in others by law.
Makino SI, Cheun HI, Watarai M, Uchida I, Takeshi K. (2001). Detection of anthrax spores from the air by real-time PCR. Lett Appl Microbiol 33: 237–240.	[Hoffmaster et al 2002]
Makino SI, IInuma-Okada Y, Maruyama T, Ezaki T, Sasakawa C, Yoshikawa M. (1993). Direct detection of *Bacillus anthracis* DNA in animals by polymerase chain reaction. Journal of Clinical Microbiology.. 31: 3, 547-551.	To establish a method for specifically detecting B. anthracis for practical applications, such as for the inspection of abattoirs, the cap region, which is essential for encapsulation in B. anthracis, was used in a DNA hybridization study by polymerase chain reaction (PCR). Oligonucleotide primers were designed to amplify a 288-bp DNA fragment within the capA gene by PCR. The amplified DNA sequence specifically hybridized to the DNA of B. anthracis but not to that of other bacterial strains tested. Since this PCR based method efficiently and specifically detected the capA sequence of bacteria in blood and spleen samples of mice within 8 h after the administration of live B. anthracis, this PCR system could be used for practical applications. By using lysis methods in preparing the samples for PCR, it was possible to amplify the 288-bp DNA segment from samples containing very few bacteria, as few as only 1 sporeforming unit; it is therefore concluded that the PCR detection method developed in this study will permit the monitoring of B. anthracis contamination in the environment.

Malkov AV. (1972). Sensitivity to antibiotics of some local strains of pathogenic bacteria. Veterinariya, Moscow. 9, 98-100.	Sensitivity to penicillin, streptomycin, chlortetracycline, oxytetracycline, monomycin, neomycin, levomycin, erythromycin and grizin was tested in 110 strains of pathogenic bacteria, including anthrax, swine erysipelas, listerellosis, pasteurellosis and salmonellosis organisms, and various diplococci, isolated from farm animals from 1966 to 1971, in the Udmurt SSR. The paper disc method was employed. The number of resistant strains was relatively low; 31 strains were resistant to streptomycin only, one strain of E. insidiosa was resistant to neomycin, and only two strains of Salmonella showed multiple antibiotic resistance. Of the 31 strains resistant to streptomycin, one was B. anthracis, three were L monocytogenes, four were pasteurellae, four were E insidiosa, 18 were salmonellae and one was Diplococcus. The high rate of streptomycin resistance (29% compared with 0.9% of neomycin resistance and 1.8% multiple antibiotic resistance) is in accordance with the widespread use of streptomycin for treating farm animals.
Malovastyi KS. (1985). Suppression of immunomorphogenesis in animals inoculated with anthrax 'STI' vaccine while being treated with antibiotics. Problemy veterinarnoi immunologii. Agropromizdat, Moscow, USSR:. 87-90.	
Mammerickx M. (1995). Vaccination trials against anthrax of the animals, from 1882 to 1884 in the area of Herve, under the direction of Pasteur. Annales de Medecine Veterinaire.. 139: 3, 147-154.	
Manchee RJ, Broster MG, Melling J, et al. (1981). *Bacillus anthracis* on Gruinard Island. Nature 294:254-255.	Nearly all viable Ba spores were found in top 6cm of soil. Adding calf blood to Ba spore containing soil and incubation at 37degC led to increase in spore numbers
Manichev AA, Shmorgun BI. (1991). Development of standard antigen from vaccine strains of *Bacillus anthracis*. Veterinariya (Moskva).. 7, 30-31.	
Marcus H, Danieli R, Epstein E, Velan B, Shafferman A, Reuveny S. (2004). Contribution of immunological memory to protective immunity conferred by a *Bacillus anthracis* protective antigen-based vaccine. Infection and Immunity. . 72: 6, 3471-3477.	Protective antigen (PA)-based vaccination is an effective countermeasure to anthrax infection. While neutralizing anti-PA antibody titers elicited by this vaccine serve as good correlates for protection against anthrax (S. Reuveny, M. D. White, Y. Y. Adar, Y. Kafri, Z. Altboum, Y. Gozes, D. Kobiler, A. Shafferman, and B. Velan, Infect. Immun. 69:2888-2893, 2001), no data are available on the contribution of the immunological memory for PA itself to protection. We therefore developed a guinea pig model in which a primary immunization with threshold levels of PA can induce a long-term T-cell immunological memory response without inducing detectable anti-PA antibodies. A revaccination of primed animals with the same threshold PA levels was effective for memory activation, yielding a robust and rapid secondary response. A challenge with a lethal dose (40 50% lethal doses; 2,000 spores) of spores after the booster vaccinations indicated that animals were not protected at days 2, 4, and 6 postboosting. Protection was achieved only from the 8th day postboosting, concomitant with the detection of protective levels of neutralizing antibody titers in the circulation. The practical implications from the studies reported herein are that, as expected, the protective capacity of memory depends on the PA dose used for the primary immunization and that the effectiveness of booster immunizations for the

	postexposure treatment of anthrax may be very limited when no detectable antibodies are present in primed animals prior to *Bacillus anthracis* spore exposure. Therefore, to allow for the establishment of memory-dependent protection prior to the expected onset of disease, booster immunizations should not be used without concomitant antimicrobial treatment in postexposure scenarios.
Marinkova VA. (1976). Efficacy of a simultaneous vaccine against anthrax, brucellosis and listeriosis in sheep. Uchenye Zapiski Kazanskogo Veterinarnogo Instituta.. 123: 35-39.	A combined vaccine against anthrax, brucellosis and listeriosis was inoculated s/c into 8 sheep. Antibody titres against Brucella and Listeria in these sheep were compared with antibody titres in sheep given single vaccines against Brucella or Listeria. Antibody titres to Listeria were about the same 7 days after vaccination in both groups (1:260-1:270) but titres declined faster in animals given the combined vaccine. Brucella antibody titres were much lower in animals given the combined vaccine.
Marinkova VV. (1976). Survival times of vaccine strains of *Bacillus anthracis*, Brucella abortus and Listeria monocytogenes in sheep simultaneously immunized against these bacteria. Uchenye Zapiski Kazanskogo Veterinarnogo Instituta.. 123: 49-52.	A combined vaccine against anthrax, brucellosis and listeriosis was inoculated s/c into 13 sheep aged 6-8 months. There was a slight, temporary increase in body temperature after vaccination. Lameness was observed in the limb where the injection was given and there was slight oedema around the injection site. Within 7-15 days after vaccination, microorganisms were isolated from the parenchymatous organs, bone marrow and brain, regional and then peripheral lymph nodes. Brucella strain 19 was still recovered from vaccinated animals after 30 days, while in most cases Listeria and *Bacillus anthracis* were isolated only during the first two weeks.
Marshall E. (1988). Sverdlovsk: anthrax capital? Science 240: 383-385	
Matras J, Mizak L, Mierzejewski J. (1999). Immunization against anthrax in animals and man. Zycie Weterynaryjne.. 74: 5, 196-197.	[Polish]
Matsune W. (2003). Direct and rapid detection of *Bacillus anthracis* DNA added to cattle blood and meat. Journal of the Japan Veterinary Medical Association.. 56: 11, 741-744.	[Japanese] *Bacillus anthracis* Pasteur II cells were added to cattle blood and meat to investigate a direct, rapid method for detecting DNA by means of nested PCR targeting the PA and capA genes. In preparing the samples for nested PCR, a cell disrupter and spin columns proved effective. It was possible to detect target DNA amplicons from 1 ml of blood containing 104 cfu and from 1 g of meat containing 105 cfu. DNA detection required only 4 h. In association with such bacteriological methods as the ascoli test, the phage test, and bacterial culture, this simple, speedy system can be useful for diagnosis of cattle anthrax.
Matyas GR, Friedlander AM, Glenn GM, Little S, Yu JM, Alving CR. (2004). Needle-free skin patch vaccination method for anthrax. Infection and Immunity. . 72: 2, 1181-1183.	Three immunizations of mice with recombinant protective antigen (rPA) by transcutaneous immunization (TCI) induced long-term neutralizing antibody titers that were superior to those obtained with aluminum-adsorbed rPA. In addition, rPA alone exhibited adjuvant activity for TCI. Forty-six weeks after completion of TCI, 100% protection was observed against lethal anthrax challenge.
Maynard JA, Maassen CB, Leppla SH, et al. (2002). Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity. Nat Biotechnol 20: 597–601.	[Scobie et al 2005, 11]
McAuliffe PR, Hardefeldt KW, Hucker DA (1978). Health problems associated with the export of live sheep. Aust Vet J 54:	Sheep were vaccinated against anthrax on assembly and before transport.

594-595	
McGee ED, Fritz DL, Ezzell JW, Newcomb HL, Brown RJ, Jaax NK. (1994). Anthrax in a dog. Veterinary Pathology.. 31: 4, 471-473.	In Mississippi, USA, in September 1991, anthrax was diagnosed in a male Labrador Retriever (6 years old) that died 2 days after initial clinical signs of ptyalism and swelling of the right forelimb. PM findings included splenomegaly, a friable liver, and blood in the stomach, small intestine and colon. A raised, haemorrhagic puncture wound (2 x 2 cm) was found on the swollen limb. The diagnosis was suspected from histopathological evidence and confirmed by electron microscopic detection of specific immunogold labelling of the cell wall and intercellular septa of a vegetative bacilli using a monoclonal antibody technique. The source of the infection was unknown. However, anthrax was reported on 26 farms within 7 Mississippi Delta counties between June and September, 1991. It was presumed that exposure had occurred during a dove hunt over freshly ploughed fields 7 days before the dog died.
McKendrick DRA. (1980). Anthrax and its transmission to humans. Cent Afr J Med;26:126-129.	
Meldrum KC. (1989a). Anthrax vaccination. Vet Rec. 125(2):48-9.	Comment in: Vet Rec. 1989 Jul 29;125(5):118. Publication Types: Letter
Meldrum KC. (1989b). Anthrax vaccination. Vet Rec. 125(6):136.	Publication Types: Letter
Merrill L, Richardson J, Kuske CR, Dunbar J. (2003). Fluorescent heteroduplex assay for monitoring Bacillus anthracis and close relatives in environmental samples. Appl Environ Microbiol 69(6): 3317-3326	Biosciences Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, USA. A fluorescent heteroduplex method was developed to assess the presence of 16S rRNA gene (rDNA) sequences from Bacillus anthracis and close relatives in PCR-amplified 16S rDNA sequence mixtures from environmental samples. The method uses a single-stranded, fluorescent DNA probe, 464 nucleotides in length, derived from a B. anthracis 16S rRNA gene. The probe contains a unique, engineered deletion such that all probe-target duplexes are heteroduplexes with an unpaired G at position 343 (deltaG343). Heteroduplex profiles of sequences >/=85% similar to the probe were produced using an ABI 377 sequencer in less than 3 h. The method divides strains of the Bacillus cereus-Bacillus thuringiensis-B. anthracis group into two subgroups. Each subgroup is defined by a specific 16S rRNA gene sequence type. Sequence type A, containing one mismatch with the probe, occurs in B. anthracis and a small number of closely related clonal lineages represented mostly by food-borne pathogenic isolates of B. cereus and B. thuringiensis. Sequence type B, containing two mismatches with the probe, is found in the majority of B. cereus and B. thuringiensis strains examined to date. Sequence types A and B, when hybridized to the probe, generate two easily differentiated heteroduplexes. Thus, from heteroduplex profiles, the presence of B. cereus-B. thuringiensis-B. anthracis subgroups in environmental samples can be inferred unambiguously. The results show that fluorescent heteroduplex analysis is an effective profiling technique for detection and differentiation of sequences representing small phylogenetic or functional groups in environmental samples.
Meselson M, Guillemin J, Hugh-Jones M, Langmuir A, Popova I, Shelokov A, Yampolskaya O. (1994). The Sverdlovsk anthrax outbreak of 1979. Science 266: 1202-1208.	
Mesnage S, Tosi-Couture E, Mock M, Fouet A. (1999). The S-layer homology domain as a means for anchoring heterologous	

proteins on the cell surface of *Bacillus anthracis*. J Appl Microbiol 87: 256-260	
Metcalfe N (2004). The history of woolsorters disease: a Yorkshire beginning with an international future? Occupational Medicine 54: 489-493	Dr JH Bell of Bradford on suggestion of Dr JE Eddison of Leeds, demonstrated in 1879 that animals inoculated with blood from a fatal case of woolsorters' disease died of anthrax. Woolsorters' disease was a feared industrial disease associated primarily with Yorkshire's textile industry of the nineteenth and early twentieth centuries. Early occupational health methods were attempted locally before concerted national efforts produced legislative measures. When its link with anthrax was established, attention in prevention focused upon chemical disinfection methods.Together, these factors were instrumental in decreasing the incidence of woolsorters' disease. However, by the beginning of the Second World War, the lack of treatment options for anthrax meant that the bacterium was experimented upon as a potential war-winning weapon.Today,woolsorters' disease and other industrial manifestations of anthrax are extremely rare, but the increasing threat of bioterrorism means that the international dread and historical lessons of this significant condition should never be forgotten. Consequently, this paper reveals the history of woolsorters' disease in order to remind those involved in occupational medicine today of the dread it caused both physicians and workers in previous generations.
Meyerhoff A, Albrecht R, Meyer JM, Dionne P, Higgins K, Murphy D. (2004). US Food and Drug Administration approval of ciprofloxacin hydrochloride for management of postexposure inhalational anthrax. Clin Infect Dis. 39(3):303-8.	US Food and Drug Administration, Rockville, MD, USA. am282@gunet.georgetown.edu In August 2000, the US Food and Drug Administration (FDA) approved ciprofloxacin hydrochloride (Cipro; Bayer) for management of postexposure inhalational anthrax. This was the first antimicrobial drug approved by the FDA for use in treating an infection due to a biological agent used intentionally. The terrorist attacks of 2001 involving anthrax underscore the imperative that safe and effective drugs to manage such infections be readily available in the United States. The approval of ciprofloxacin hydrochloride, which was made on the basis of a surrogate human marker of efficacy, made extensive use of data from an animal model of disease. This represents a new direction in the development of efficacy data in support of drug approval and facilitates the availability of those drugs for which there is an urgent need. This article presents the scientific data and regulatory mechanism that supported the approval of ciprofloxacin hydrochloride for management of postexposure of inhalational anthrax.
Michel C, Brouillaud J, Poulet P. (1973). [Differential diagnosis of *Bacillus anthracis* and Bacillus cereus by immunodiffusion]. Bulletin de l'Office International des Epizooties.. 79: 3/4, 289-296.	
Michel C, Poussot A, Chabassol C, Foata D, Poulet P. (1973). [Rapid detection of *Bacillus anthracis* by immunofluorescence]. Bulletin de l'Academie Veterinaire de France.. 46: 8, 333-342.	The advantages of the immunofluorescence reaction in the microscopical diagnosis of anthrax are discussed. Cross-reactions exist between B. anthracis and B. cereus. Immunofluorescence, direct and indirect, gives rapid identification of the vegetative form of B. anthracis, provided optimum dilutions of immune serum are used. Preliminary absorption of the immune serum with heterologous strains of B. cereus, megaterium and subtilis increases the range of specificity of the lowest dilutions. The antigenic relationship between B. anthracis and B. cereus is due to the presence of mucopeptides and lipoproteins common to both. The absorption of immunoglobulins does not allow the differentiation of the spores of B. cereus, which appear as fluorescent as those of B. anthracis.
Mikhailyuk AP, Barsegyan BS, Pronin IA, Avetisyan AV, Kadymov RA, Voskanyan GE. (1982). Combined and simultaneous	

vaccination of animals (against two or more diseases--a brief review). Veterinariya, Moscow.. 12, 51-52.	
Mikszta JA, Sullivan VJ, Dean C, Waterston AM, Alarcon JB, Dekker JP 3rd, Brittingham JM, Huang J, Hwang CR, Ferriter M, Jiang G, Mar K, Saikh KU, Stiles BG, Roy CJ, Ulrich RG, Harvey NG. (2005). Protective immunization against inhalational anthrax: a comparison of minimally invasive delivery platforms. J Infect Dis. 191(2): 278-288	BD Technologies, 21 Davis Dr., Research Triangle Park, NC 27709, USA. john_mikszta@bd.com A new anthrax vaccine under clinical investigation is based on recombinant *Bacillus anthracis* protective antigen (rPA). Here, we investigated microneedle-based cutaneous and nasal mucosal delivery of rPA in mice and rabbits. In mice, intradermal (id) delivery achieved up to 90% seroconversion after a single dose, compared with 20% after intramuscular (im) injection. Intranasal (inl) delivery of a liquid formulation required 3 doses to achieve responses that were comparable with those achieved via the id or im routes. In rabbits, id delivery provided complete protection against aerosol challenge with anthrax spores; in addition, novel powder formulations administered inl provided complete protection, whereas a liquid formulation provided only partial protection. These results demonstrate, for the first time, that cutaneous or nasal mucosal administration of rPA provides complete protection against inhalational anthrax in rabbits. The novel vaccine/device combinations described here have the potential to improve the efficacy of rPA and other biodefense vaccines.
Milanovic A. (1982). Infectious diseases of dairy cattle and milk production. Veterinaria, Yugoslavia.. 31: 3/4, 433-439.	Viral and bacterial diseases that may be transmitted to consumers via milk are discussed, details being given of Yugoslav regulations defining the conditions under which milk should be classed as totally unfit for human consumption and the conditions under which it should be considered as fit for human consumption only after heat treatment. In 1980, milk from 1104 cows in Yugoslavia was classed in one or the other of these categories, because of rabies (933 cases), tuberculosis (138) or anthrax (33).
Mi-Li Gu; Leppla SH, Klinman DM. (1999). Protection against anthrax toxin by vaccination with a DNA plasmid encoding anthrax protective antigen. Vaccine.. 17: 4, 340-344.	A DNA vaccine encoding the immunogenic and biologically active portion of anthrax protective antigen (PA) was constructed. Spleen cells from BALB/c mice immunized intramuscularly with this vaccine were stimulated to secrete IFN gamma and IL-4 when exposed to PA in vitro. Immunized mice also mounted a humoral immune response dominated by IgG1 anti-PA antibody production, the subclass previously shown to confer protection against anthrax toxin. A 1:100 dilution of serum from these animals protected cells in vitro against cytotoxic concentrations of PA. Moreover, 7/8 mice immunized three times with the PA DNA vaccine were protected against lethal challenge with a combination of anthrax protective antigen plus lethal factor.
Millar H (2003). Anthrax in animals. Agriculture notes, Department of Primary Industries, Victoria, AG0802	
Miller CJ, Elliott JL, Collier RJ. (1999). Anthrax protective antigen: prepore to ore conversion. Biochemistry 38: 10432-41	
Miller ES, Scott EB, Noe HA, Madin SH, Henley TF (1946). Chemotherapy of experimental anthrax infections. J Immunol 53: 371-379	
Miller M, Roche P, Yohannes K, Spencer J, Bartlett M, Brotherton J, Hutchinson J, Kirk M, McDonald A, Vadjic C. (2005).	In 2003, fifty eight diseases and conditions were notifiable at a national level in Australia. States and territories reported a total of 104 956 cases to the National Notifiable Diseases Surveillance System an increase of 3.2% on the total number of notifications in 2002. In 2003, the most frequently notified diseases were sexually acquired infections (38

Australia's notifiable diseases status, 2003 annual report of the National Notifiable Diseases Surveillance System. Communicable Diseases Intelligence. 29: 1, 1-61.	854, 37% of total notifications; chlamydial infection, donovanosis, gonococcal infection, syphilis, syphilis-congenital), gastrointestinal diseases (24 655 notifications, 24%; botulism, campylobacteriosis, cryptosporidiosis, hepatitis A, hepatitis E, listeriosis, salmonellosis (non-typhoidal), shigellosis, shiga-like toxin producing/verotoxigenic Escherichia coli, haemolytic uraemic syndrome, typhoid) and bloodborne viruses (20 825 notifications, 20%; hepatitis B, hepatitis C, hepatitis D). There were 11 113 notifications of vaccine preventable diseases (diphtheria, Haemophilus influenzae type b disease, influenza (laboratory confirmed), measles, mumps, pneumococcal disease (invasive), poliomyelitis, rubella, tetanus), 6780 notifications of vectorborne diseases (Barmah forest virus infection, Ross river virus infection), 1826 notifications of other bacterial infections (legionellosis, leprosy, invasive meningococcal disease, laboratory-based meningococcal surveillance, tuberculosis) and 903 notifications of zoonotic diseases (anthrax, Australian bat lyssaviral and lyssaviral (unspecified) infections, leptospirosis, ornithosis, Q fever).
Minett FC (1950). Sporulation and viability of B. anthracis in relation to environmental temperature and humidity. Journal of Comparative Pathology 60:161–176.	
Mishra U, Pradhan A, Singh UM. (1993). Incidence of anthrax in Kathmandu valley. Veterinary Review (Kathmandu).. 8: 1, 26-28.	Sudden death and oozing of unclotted blood from natural orifices of animals have been reported in Nepal. Anthrax was confirmed for the first time in December 1992 following an outbreak in 4 cattle in Kathmandu valley. In subsequent months anthrax was diagnosed in a further 20 cattle, 4 horses and 2 pigs that had died. Antibiotics were tried in the last moment without success but prompt vaccination of all in contact animals with live attenuated vaccine prevented further outbreaks.
Misra RP. (1991). Manual for the production of anthrax and blackleg vaccines. Manual for the production of anthrax and blackleg vaccines.. Food and Agriculture Organization, Rome, Italy	A technical manual on the preparation of anthrax spore vaccine and adsorbed vaccine against Clostridium chauvoei.
Mizak L. (2004). Anthrax--continuous threat to humans and animals Przegl Epidemiol. 58(2):335-42.	Osrodek Diagnostyki i Zwalczania Zagrozen Biologicnych, Wojskowy Instytut Higieny i Epidemiologii w Pulawach. Gram-positive, spore-forming, aerobic bacterium *Bacillus anthracis* is an etiological agent of anthrax a disease very dangerous to humans and all warm-blooded animals. The spore forms are markedly resistant to unfavourable environmental extremes of heat, cold, desiccation, chemicals, irradiation etc. The vegetative forms characterised virulence factors: the antiphagocytic poly-gamma-D-polipeptide capsule and three proteins, edema factor (EF), lethal factor (LF) and protective antigen (PA). Anthrax is mainly transmitted from animals to man through food of animal origin, animal products and contamination of the environment with B. anthracis and its spores. There are three types of this disease: cutaneous, intestinal and inhalation anthrax. Research on anthrax as a biological weapon began more then 80 years ago. Depending on the target chosen and the scale of the attack the anthrax spores may by used to contaminate of foodstuffs or liquids and water. The aerosolised release of anthrax spore can cause illness with a high fatality rate.
Moayeri M, Leppla SH (2004). The roles of anthrax toxin in pathogenesis. Curr Opinion in Microbiology 7: 19-24	
Mock M, Fouet A. (2001). Anthrax. Annual Review of Microbiology.. 55: 647-671.	*Bacillus anthracis* was shown to be the aetiological agent of anthrax by R. Koch and L. Pasteur at the end of the 19th century. The concepts on which medical microbiology are based arose from their work on this bacterium. The link

	between plasmids and major virulence factors of B. anthracis was not discovered until the 1980s. The three toxin components are organized in two A-B type toxins, and the bacilli are covered by an antiphagocytic polyglutamic capsule. Structure-function analysis of the toxins indicated that the common B-domain binds to a ubiquitous cell receptor and forms a heptamer after proteolytic activation. One enzyme moiety is an adenylate cyclase and the other is a Zn2+ metalloprotease, which is able to cleave MAPKKs. The capsule covers an S-layer sequentially composed of two distinct proteins. Knowledge of the toxins facilitates the design of safer veterinary vaccines. Spore-structure analysis could contribute to the improvement of human nonliving vaccines. The phylogeny of B. anthracis within the Bacillus cereus group is also reviewed.
Mock M, Levy M, Brossier F. (2003). History and future of vaccine development against anthrax. (NATO Science Series: Life and Behavioural Sciences Volume 352) Applications of genomics and proteomics for analysis of bacterial biological warfare agents: Proccedings of the NATO Advanced Workshop held in Bratislava, Slovak Republic, 24-28 July 2002. IOS Press, Amsterdam, Netherlands:. 129-134.	Anthrax affects all mammals including man. After entry into the host, the spores of *Bacillus anthracis* germinate and yield capsulated, toxin producing bacilli. The live spore vaccine, the toxinogenic non-capsulated attenuated Sterne strain, is used satisfactorily for veterinary purposes, but has side effects in some sensitive species due to residual virulence. The development of recombinant genetics of B. anthracis has led to the appearance of novel alternatives. We have constructed Sterne-strain derivatives producing genetically detoxified oedema factor (EF) and lethal factor (LF). These offer safer, non-toxic, live vaccine candidates. Moreover, the use of such strains would also highlight the potential of B. anthracis to deliver foreign antigens in vivo. Various strategies have been used to induce specific protective immunity. Protective antigen (PA) is the component targeted by the protective immune response, and a cell-free-PA based vaccine is used for human vaccination. However, its efficacy is far below that of the live vaccine when tested in animal models. It therefore appears that other components and/or various immune response mechanisms are probably required for efficient protection. We have shown that the addition of formaldehyde-inactivated spores (FIS) of the Sterne strain protects 100% of mice and guinea pigs against challenge with virulent B. anthracis strains under conditions in which PA alone is ineffective. Infection by B. anthracis involves both spore germination and subsequent vegetative cell multiplication. The protection conferred by FIS may act on either of these processes. We constructed a challenge strain whose virulence is entirely due to its multiplication properties. This strain is derived from the wild-type virulent strain and carries a non-polar deletion into the pagA gene encoding PA. Immunization with FIS or with PA+FIS significantly protected against infection with this capsulated PA deficient strain. The respective contribution of the immune response to PA and of spore antigens in conferring protection is discussed.
Mock M, Roques BP. (2002). Progress in rapid screening of *Bacillus anthracis* lethal factor activity PNAS 99: 6527-6529.	
Mogridge J, Cunningham K, Collier RJ (2002). Stoichiometry of anthrax toxin complexes. Biochemistry 41: 1079-1082	
Mogridge J, Cunningham K, Lacy DB, Mourez M, Collier RJ. (2002). The lethal and edema factors of anthrax toxin bind only to oligomeric forms of the protective antigen. PNAS 99: 7045-7048	
Mohamed N, Li J, Ferreira CS, Little SF, Friedlander AM, Spitalny GL, Casey LS.	We investigated the ability of using monoclonal antibodies (MAbs) against anthrax protective antigen (PA), an anthrax exotoxin component, to modulate exotoxin cytotoxic activity on target macrophage cell lines. Anthrax PA plays a critical

(2004). Enhancement of anthrax lethal toxin cytotoxicity: a subset of monoclonal antibodies against protective antigen increases lethal toxin-mediated killing of murine macrophages. Infection and Immunity. . 72: 6, 3276-3283.	role in the pathogenesis of *Bacillus anthracis* infection. PA is the cell-binding component of the two anthrax exotoxins: lethal toxin (LeTx) and edema toxin. Several MAbs that bind the PA component of LeTx are known to neutralize LeTx-mediated killing of target macrophages. Here we describe for the first time an overlooked population of anti-PA MAbs that, in contrast, function to increase the potency of LeTx against murine macrophage cell lines. The results support a possible mechanism of enhancement: binding of MAb to PA on the macrophage cell surface stabilizes the PA by interaction of MAb with macrophage Fc gamma receptors. This results in an increase in the amount of PA bound to the cell surface, which in turn leads to an enhancement in cell killing, most likely due to increased internalization of LF. Blocking of PA-receptor binding eliminates enhancement by MAb, demonstrating the importance of this step for the observed enhancement. The additional significance of these results is that, at least in mice, immunization with PA appears to elicit a poly-clonal response that has a significant prevalence of MAbs that enhance LeTx-mediated killing in macrophages.
Mohammed MJ, Marston CK, Popovic T, Weyant RS, Tenover FC. (2002). Antimicrobial susceptibility testing of *Bacillus anthracis*: comparison of results obtained by using the National Committee for Clinical Laboratory Standards broth microdilution reference and Etest agar gradient diffusion methods. J Clin Microbiol. 40(6):1902-7.	Epidemiology and Laboratory Branch, Division of Healthcare Quality Promotion. Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA. We determined the patterns of antimicrobial susceptibility of 65 isolates of *Bacillus anthracis* (50 historical and 15 recent U.S. clinical isolates) to nine antimicrobial agents using the National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution reference method. The results for the 50 historical B. anthracis isolates obtained by the broth microdilution method were compared to those generated by the Etest agar gradient diffusion method. One isolate of B. anthracis was beta-lactamase positive and resistant to penicillin (MIC, 128 microg/ml); a second isolate, which was beta-lactamase negative, was borderline penicillin resistant, with the penicillin MICs for the isolate varying from 0.12 to 0.25 microg/ml; and the remainder of the isolates were beta-lactamase negative and penicillin susceptible (MICs, <or=0.12 microg/ml). Approximately 78% of the isolates showed reduced susceptibility to ceftriaxone (MICs, >or=16 microg/ml). All B. anthracis isolates were susceptible to chloramphenicol (MICs, <or=8 microg/ml), ciprofloxacin (MICs, <or= 1 microg/ml), clindamycin (MICs, <or=0.5 microg/ml), rifampin (MICs, <or=0.5 microg/ml), tetracycline (MICs, <or=0.06 microg/ml), and vancomycin (MICs, <or=2 microg/ml) by use of NCCLS breakpoints for staphylococci. All 15 recent B. anthracis isolates from the United States were susceptible to penicillin, doxycycline, and ciprofloxacin. By use of the susceptibility breakpoint for staphylococci of <or=0.5 microg/ml, 97% of the B. anthracis isolates tested would have been categorized as intermediate to erythromycin. No statistically significant difference was found between the results of broth microdilution testing and the results of the Etest method for any of the antimicrobial agents tested; however, the results for penicillin obtained by the Etest were 1 to 9 dilutions lower than those obtained by the broth microdilution method. The differences in the penicillin MICs by the Etest method and the difficulties of reading the Etest results through the glass of a biological safety cabinet may limit the utility of this alternate susceptibility testing method for B. anthracis isolates.
Montecucco C. (2001). Detoxification of a bacterial toxin by the toxin itself. Trends in Pharmacological Sciences.. 22: 10, 493-494.	Several bacterial protein toxins act by forming oligomers on the cell surface. A novel approach to the prevention of the damage caused by oligomeric pore-forming toxins has recently been discovered using one such protein: the protective antigen (PA) of *Bacillus anthracis*. Some PA mutants act as dominant-negative effectors of the function performed by the oligomer. In fact, the PA mutant co-oligomerizes with native PA, but the mixed oligomer is non-functional. This finding might turn out to be of general validity.
Morens DM (2002). Epidemic anthrax in the eigtheenth century, the Americas. Emerg Infect Dis 8: 1160-1162	Anthrax has been described as a veterinary disease of minor importance to clinical medicine, causing occasional occupational infections in single cases or clusters. Its potential for rapid and widespread epidemic transmission under natural circumstances has not been widely appreciated. A little-known 1770 epidemic that killed 15,000 people in Saint-

	Domingue (modern Haiti) was probably intestinal anthrax. The epidemic spread rapidly throughout the colony in association with consumption of uncooked beef. Large-scale, highly fatal epidemics of anthrax may occur under unusual but natural circumstances. Historical information may not only provide important clues about epidemic development but may also raise awareness about bioterrorism potential.
Mourez M, Yan M, Lacy DB, et al. (2003). Mapping dominant-negative mutations of anthrax protective antigen by scanning mutagenesis. Proc Natl Acad Sci USA 100: 13803–8.	[Scobie et al 2005, 13]
Moynihan WA. (1963). Anthrax in Canada. Can Vet J 4: 283	
Muller JD, Wilks CR, O'Riley KJ, Condron RJ, Bull R, Mateczun A. (2004). Specificity of an immunochromatographic test for anthrax. Australian Veterinary Journal.. 82: 4, 220-222.	Objective: To evaluate the specificity of an immunochromatographic test (ICT) for anthrax in cattle. Design: A comparison of an ICT with blood smear and culture in uninfected cattle. Procedure: Two hundred and forty blood samples were collected from dead cattle a two knackeries within Victoria and tested on-site with an ICT for the detection of protective antigen (PA) of *Bacillus anthracis*. Blood smears were prepared on-site and blood samples transported to the laboratory for aerobic and anaerobic culture. The results of the ICT were compared with blood smear and culture. Animals were regarded as not infected with B. anthracis if the organism was not detected in a stained blood smear or on culture. Ten healthy yearling cattle were vaccinated with live spore anthrax vaccine and blood samples collected on days 0 to 7 and day 15 were tested in the ICT for the presence of PA. Results: All blood samples from the 240 knackery cattle were ICT, smear and culture negative. All blood samples from the 10 vaccinated cattle were ICT negative. Conclusion: The ICT is a test with high specificity in cattle (98.5 to 100%; 95% CI) and recent vaccination of cattle does not give rise to positive reactions.
Murphy FD, LaBocetta AC, Lockwood JA (1944). Treatment of human anthrax with penicillin. JAMA 126: 948-950	3 female wool workers with cutaneous anthrax successfully treated
Murray G. (1997). Unusual outbreak of anthrax in Victoria, Australia. Bull Off Int Epizoot 109(2): 143-149	The first case of anthrax in this outbreak was diagnosed on a dairy farm near Tatura on 26 January 1997. This was the first record in this area since recording of anthrax cases began in 1914. By 13 March the disease had been reported on 83 farms, with the peak on 15 February when 10 new cases appeared. Epidemiological details and control measures are described. The outbreak was associated with an unusually prolonged period of hot, dry weather conditions. Anthrax was confirmed in 200 cattle and 4 sheep. As part of the control measures, cattle and sheep were vaccinated on 590 farms (78 649 cattle, 2663 sheep).
Musa MT, Shomein AM, Abd El Razig YM, Meki NT, El-Hassan SM. (1993). Anthrax in humans and camels in the Sudan with reference to the disease in the country. Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux.. 46: 3, 438-439.	An outbreak of anthrax in February, 1988, near Kulbus town and the Chad border affected 6 dromedaries and 10 people. The 10 people had participated in the slaughter, cooking or eating of an infected dromedary; 5 died despite antibiotic treatment. Control was by mass vaccination of the animals in the area.
Muscillo M, La Rosa G, Sali M, De Carolis	

E, Adone R, Ciuchini F, Fasanella A (2005). Validation of a pXO2-A PCR Assay To Explore Diversity among Italian Isolates of *Bacillus anthracis* Strains Closely Related to the Live, Attenuated Carbosap Vaccine. Journal of Clinical Microbiology 43: 4758-4765	
Naimanov PI, Golubinskii EP, Sorkin YuI. (1984). Nutritional requirements of *Bacillus anthracis*, particularly the growth of vaccine strain STI-1 upon periodic culture in synthetic liquid media. Zhurnal Mikrobiologii Epidemiologii i Immunobiologii.. 6, 55-59.	The Russian vaccine strain and field strains required valine for growth in a defined synthetic medium. The optimum concentration of glucose for batch culture varied with the culture medium. Pyruvate and gluconate were utilized more efficiently than glucose.
Nass M (2005). A Brief History of Anthrax Vaccine Development http://www.mercola.com/article/anthrax/anthrax_warfare.htm	
Nass M: (1992). Anthrax epizootic in Zimbabwe, 1978 -- 1980: due to deliberate spread? Physicians for Social Responsibility Quarterly 2:198-209.	
Nassi S, Collier RJ, Finkelstein A (2002). PA63 channel of anthrax toxin: an extended beta-barrel. Biochemistry 41: 1445-1450	
Navas E. (2002). Problems associated with potential massive use of antimicrobial agents as prophylaxis or therapy of a bioterrorist attack. Clinical Microbiology and Infection.. 8: 8, 534-539.	In addition to the direct sanitary damage of a terrorist attack caused by biological weapons, the consequences of the massive stockpiling and consumption of antimicrobial agents in order to treat or prevent the disease under a potential epidemic due to pathogenic bacteria must also be considered. *Bacillus anthracis*, Francisella tularensis and Yersinia pestis are the bacteria most likely to be used as terrorist weapons. Tetracyclines, quinolones and aminoglycoside are the antibiotics of choice against these microorganisms. The recent terrorist attack with anthrax spores in the USA caused a substantial increase in the sales of ciprofloxacin, as thousands of citizens received antibiotic prophylaxis for either confirmed or suspected exposure to anthrax, and many others stockpiled antibiotic supplies at their homes under a panic scenario. The massive consumption of antimicrobial drugs may lead to the selection of antibiotic resistant strains, and to the appearance of undesirable side effects, such as anaphylaxis or teratogenesis. National health authorities must develop realistic protocols in order to detect, treat and prevent mass casualties caused by biological weapons. An antibiotic stockpile has to be planned and implemented, and home stockpiling of antibiotics must be strongly discouraged.

Nicholson WL, Galeano B. (2003). UV resistance of *Bacillus anthracis* spores revisited: validation of Bacillus subtilis spores as UV surrogates for spores of B. anthracis Sterne. Applied and Environmental Microbiology. . 69: 2, 1327-1330.	Recent bioterrorism concerns have prompted renewed efforts towards understanding the biology of bacterial spore resistance to radiation with a special emphasis on the spores of *Bacillus anthracis*. A review of the literature revealed that B. anthracis Sterne spores may be three to four times more resistant to 254-nm-wavelength UV than are spores of commonly used indicator strains of Bacillus subtilis. To test this notion, B. anthracis Sterne spores were purified and their UV inactivation kinetics were determined in parallel with those of the spores of two indicator strains of B. subtilis, strains WN624 and ATCC 6633. When prepared and assayed under identical conditions, the spores of all three strains exhibited essentially identical UV inactivation kinetics. The data indicate that standard UV treatments that are effective against B. subtilis spores are likely also sufficient to inactivate B. anthracis spores and that the spores of standard B. subtilis strains could reliably be used as a biodosimetry model for the UV inactivation of B. anthracis spores.
Nicoll A, Maynard R (2004). One hundred years of anthrax. From wool-sorters' to mail-sorter' disease. Occup Environ Med 61: 95	Editorial re history of woolsorters' disease at Kiddermaster in the UK in the early 1900s and lessons for current anthrax incidents. Notes that in addition to biological agents, terrorist groups may utilise chemical and radioactive materials.
Nidhi Ahuja; Praveen Kumar; Rakesh Bhatnagar. (2001). Rapid purification of recombinant anthrax-protective antigen under nondenaturing conditions. Biochemical and Biophysical Research Communications.. 286: 1, 6-11.	This paper presents a rapid and efficient strategy for purification of recombinant anthrax-protective antigen that involves ion exchange and hydrophobic interaction chromatography. This method allows 3 mg of protective antigen to be purified to homogeneity from a 1-litre culture in just 6 hours and ensures that there is no loss of activity and that the conformational integrity of the epitopes is not disturbed. The results presented may be used for the development of a safe and efficacious recombinant vaccine against anthrax.
Nidhi Ahuja; Praveen Kumar; Sheeba Alam; Megha Gupta; Rakesh Bhatnagar. (2003). Deletion mutants of protective antigen that inhibit anthrax toxin both in vitro and in vivo. Biochemical and Biophysical Research Communications.. 307: 3, 446-450.	The anthrax toxin complex is primarily responsible for most of the symptoms of anthrax. This complex is composed of three proteins, anthrax protective antigen, anthrax oedema factor, and anthrax lethal factor. The three proteins act in binary combination of protective antigen plus oedema factor (oedema toxin) and protective antigen plus lethal factor (lethal toxin) that paralyze the host defenses and eventually kill the host. Both oedema factor and lethal factor are intracellularly acting proteins that require protective antigen for their delivery into the host cell. In this study, we show that deletion of certain residues of protective antigen results in variants of protective antigen that inhibit the action of anthrax toxin both in vitro and in vivo. These mutants protected mice against both lethal toxin and oedema toxin challenge, even when injected at a 1:8 ratio relative to the wild-type protein. Thus, these mutant proteins are promising candidates that may be used to neutralize the action of anthrax toxin.
Niebuhr SE, Dickson JS. (2003). Destruction of *Bacillus anthracis* strain Sterne 34F2 spores in postal envelopes by exposure to electron beam irradiation. Letters in Applied Microbiology. . 37: 1, 17-20.	Aims: To determine the irradiation dose necessary to reduce the populations of *Bacillus anthracis* spores in a dry medium in postal envelopes. Methods and Results: *Bacillus anthracis* Sterne 34F2 spores were dispersed in non-fat dry milk and then placed into standard business postal envelopes. The spores were treated with a sequence of irradiation doses to determine the decimal reduction value (D10) in kiloGrays (kGy). The average D10 value was 3.35+or-0.02 kGy. Conclusions: An irradiation dose of 40.2 kGy would be required to result in a process equivalent to the thermal canning process (12 D10 reduction) to eliminate Clostridium botulinum spores. Significance and Impact of the Study: Irradiation is an effective means of reducing or eliminating B. anthracis spores in a dry medium in postal envelopes.
Nikanorov BA, Kolesov SG, Kretilin VK, Solov'ev LB, Polyakov VA, Belkina RN, Mitin SS. (1976). Preparation of anthrax	

vaccine using culture apparatus "AKM-Sh". Veterinariya, Moscow.. 12, 28-29.	
Nishi JS, Dragon DC, Elkin BT, Mitchell J, Ellsworth TR, Hugh-Jones ME. (2002). Emergency response planning for anthrax outbreaks in bison herds of northern Canada: a balance between policy and science. Ann N Y Acad Sci. 969:245-50.	Resources, Wildlife, and Economic Development (RWED), Government of the Northwest Territories (GNWT), Fort Smith, Northwest Territories X0E 0P0, Canada. John_Nishi@gov.nt.ca\ Anthrax outbreaks in northern Canada have implications for ongoing recovery efforts for the threatened wood bison and may pose a health risk to humans, other wildlife, and domestic livestock. RWED and WBNP maintain Anthrax Emergency Response Plans (AERPs) for their respective jurisdictions. An AERP is a pre-planned logistical framework for responding effectively and rapidly to an outbreak so as to minimize spread of the disease, reduce environmental load of spores available for future outbreaks, and minimize risk to public health. In this paper, we describe the main components of an AERP and outline areas for future research. Carcase disposal: lime and burial not useful. Lime provides favourable pH and burial provides a potential reservoir.
Nizamov RN. (1982). Phenotypic variation induced in *Bacillus anthracis* by chemical agents, [formaldehyde, monochloroacetic acid, hydrogen peroxide, fenosmolin]. Razrabotka effektivnykh metodov profilaktiki i lecheniya zhivotnykh pri infektsionnykh zabolevannyakh. (Development of effective methods for preventing and treating infectious diseases of animals).. Veterinarnyi Institut, Kazan, USSR:. 93-101.	When the "STI" vaccine strain, Tsenkovskii's 2nd vaccine strain and the weakly virulent, encapsulated strain "Ch" of B. anthracis were exposed to subsporicidal concentrations of the disinfectants, they underwent dissociation, with alteration of carbohydrate metabolism, reduction of virulence and weakening of immunogenicity.
Noah DL, Noah DL, Crowder HR. (2002). Biological terrorism against animals and humans: a brief review and primer for action. J Am Vet Med Assoc. 221(1):40-3.	Office of the Air Force Surgeon General Headquarters, United States Air Force, Bolling Air Force Base, Washington, DC 20330, USA.
Noseda R, Mock M, Levy M, Cordeviola JM, Fiscalini B, Bigalli C, Combessies GM, Martinez AH, Bardon JC, Acuna CM. (2002). Anthrax in man through infection from cattle: diagnosis by traditional methods and molecular characterization (PCR). Veterinaria Argentina.. 19: 188, 581-590.	An outbreak of rural anthrax is described, with 25 cattle deaths and cutaneous carbuncle in 2 men. The 304 cattle in the herd had been vaccinated with a Sterne strain spore vaccine 4 months previously. Possible environmental predisposing extrinsic and intrinsic causes were evaluated. *Bacillus anthracis* strains from cattle, human, vaccinal and soil-borne origin were isolated and identified, employing traditional microbiological and new identification methods by genetic amplification (PCR). Antibiotic sensitivity was determined. New techniques for the disposal of carcasses by Controlled Burial were implemented and evaluated.
Noseda RP, Combessies G, Orella P, Cordeviola JM, Fiscalini B, Bigalli C, Bardon JC, Martinez AH. (2004). Respiratory bovine anthrax. Veterinaria Argentina.. 21: 207, 506-512.	A case of respiratory bovine anthrax which occurred in a cattle farm in Tapalque, Buenos Aires Province, is described. The animal, which had shown respiratory difficulties previous to its death, was a 15 month old cow Aberdeen Angus breed from a lot of 52 bovines that were not vaccinated against anthrax. At necropsy, the congested lungs, with many petechial haemorrhages, and the mediastinal ganglion with haemorrhagic lymphoadenitis were observed. A quick ELISA test allowed us to show Protective Antigen (PA) using the tracheo-bronchial secretion. The bacterial cultures allowed us to isolate and typify *Bacillus anthracis* and to determine the antibiotic sensitiveness. Mice inoculated with

	such a bacterial strain died within 32 hours. At necropsy, the hepatomegaly was obvious, from which the inoculated bacterial strain could be reisolated. Cattle vaccination and "controlled covering" of the carcasses are suggested methods for a major control of the disease.
Noseda RP, Cordeviola JM, Fiscalini B, Bardon JC, Martinez AH, Combessies GM. (2001). Bovine anthrax: epidemiological survey of 46 outbreaks in the province of Buenos Aires and their relationship with human diseases. Veterinaria Argentina.. 18: 178, 578-587.	46 outbreaks of bovine anthrax occurred in Buenos Aires province during 1990-2000. Anthrax caused 14% of sudden death cases annually between 1977 and 2000. In this period, 2018 bone marrow samples with presumptive diagnosis of anthrax were analysed. *Bacillus anthracis* was isolated and identified in 279 cases. 57% of outbreaks occurred when the weather was wet, 62% of affected animals grazed on natural fields and 72% grazed on high grass. There were streams on 52% of affected farms. 5% of affected animals died of anthrax. Adult animals were mostly affected (84%). Animals on 72% of farms were unvaccinated. Most important postmortem findings were sudden death (98%), blood exudation (78%) and enlarged spleen (67%) and 57% of corpses had been attacked by prey birds. Seven people were also affected. All of them had contacts with dead animals. Six people had cutaneous anthrax and one person had gastrointestinal anthrax which was lethal.
Novak JS, Call J, Tomasula P, Luchansky JB. (2005). An assessment of pasteurization treatment of water, media, and milk with respect to Bacillus spores. Journal of Food Protection. . 68: 4, 751-757.	This study evaluated the ability of spore-forming Bacillus spp. to resist milk pasteurization conditions from 72 to 150 degrees C. Spores from the avirulent surrogate Sterne strain of *Bacillus anthracis*, as well as a representative strain of a common milk contaminant that is also a pathogen, Bacillus cereus ATCC 9818, were heated at test temperatures for up to 90 min in dH2O, brain heart infusion broth, or skim milk. In skim milk, characteristic log reductions (log CFU per milliliter) for B. anthracis spores were 0.45 after 90 min at 72 degrees C, 0.39 after 90 min at 78 degrees C, 8.10 after 60 min at 100 degrees C, 7.74 after 2 min at 130 degrees C, and 7.43 after 0.5 min at 150 degrees C. Likewise, log reductions (log CFU per milliliter) for viable spores of B. cereus ATCC 9818 in skim milk were 0.39 after 90 min at 72 degrees C, 0.21 after 60 min at 78 degrees C, 7.62 after 60 min at 100 degrees C, 7.37 after 2 min at 130 degrees C, and 7.53 after 0.5 min at 150 degrees C. No significant differences ($P<0.05$) in thermal resistance were observed for comparisons of spores heated in dH2O or brain heart infusion broth compared with results observed in skim milk for either strain tested. However, spores from both strains were highly resistant ($P<0.05$) to the pasteurization temperatures tested. As such, pasteurization alone would not ensure complete inactivation of these spore-forming pathogens in dH2O, synthetic media, or skim milk.
Nulens E, Voss A. 2002 Laboratory diagnosis and biosafety issues of biological warfare agents. Clinical Microbiology and Infection. . 8: 8, 455-466.	Bioterrorism events have been rare until recently. Many clinical laboratories may not be familiar with handling specimens from a possible bioterrorism attack. Therefore, they should be aware of their own responsibilities and limitations in the handling and treatment of such specimens, and what to do if they are requested to process clinical samples. The Centers for Disease Control and Prevention has developed the Laboratory Response Network to provide an organized response system for the detection and diagnosis of biological warfare agents based on laboratory testing abilities and facilities. There are potentially many biological warfare agents, but probably a limited number of agents would be encountered in case of an attack, such as *Bacillus anthracis*, Brucella spp., Clostridium botulinum, Yersinia pestis, Francisella tularensis and viruses causing smallpox and viral haemorrhagic fevers, and their identification and laboratory safety will be discussed.
Nungester WJ. (1967). Problems for future study of anthrax. Federation Proceedings 26: 1571.	15 problems presented, including active immunization of wildlife by aerosol, improved human vaccines, better disinfection of environments, improved treatments
O'Brien J, Friedlander A, Dreier T, Ezzell J, Leppla S (1985). Effects of anthrax toxin	[During et al 2001, 7]

components on human neutrophils. Infect Immun 47: 306–10.	
Odarenko KI, Zhanuzakov NZh, Zadorozhnyi IF, Beisenov BB, Il'yasov BK, Dutov MM, Kasenov SK. (1978). Combined vaccine against anthrax and blackleg. Veterinariya, Moscow. 7, 49-51.	
Odendaal MW, Pieterson PM, de Vos V, Botha AD. (1991). The antibiotic sensitivity patterns of *Bacillus anthracis* isolated from the Kruger National Park. Onderstepoort J Vet Res. 58(1):17-9.	Roodeplaat Research Laboratories, Sinoville. Forty-four isolates of *Bacillus anthracis* made from carcasses and soil in different localities of an endemic anthrax area in the Kruger National Park, South Africa, were tested by standard disc diffusion for their susceptibility to 18 different antibiotics. These were ampicillin, penicillin G, sulphatriad, streptomycin, clindamycin, gentamicin, fusidic acid, trimethoprim, sulphamethoxazole, chloramphenicol, erythromycin, methicillin, tetracycline (2 different concentrations), novobiocin, cefotaxime, netilmicin, cefamandole and cefoxitin. All the isolates were susceptible to ampicillin, streptomycin, chloramphenicol, erythromycin, tetracycline, methicillin and netilmicin. More than 90% of the isolates were sensitive to clindamycin, gentamicin and cefoxitin, whereas only 84.1% of the isolates were sensitive to penicillin G, 86.4% to novobiocin and 68.18% to cefamandole. Complete resistance in 100% of the isolates was encountered with trimethoprim and sulphamethoxazole, with 95.45% for sulphatriad. Moderate sensitivity occurred with penicillin G (15.9% of the isolates), clindamycin (6.8%), novobiocin (13.6%), fusidic acid (84.1%), cefotaxime (100%), cefamandole (31.8%) and cefoxitin (6.8%). The relevance of the findings to the therapeutic uses of different types of antibiotic in human clinical cases referred to in the literature is discussed.
Oggioni MR, Ciabattini A, Cuppone AM, Pozzi G. (2003). Bacillus spores for vaccine delivery. Vaccine.. 21: Supplement 2, S2/96-S2/101.	Spores of the genus Bacillus have been used for a long time as probiotics for oral bacteriotherapy both in humans and in animals. Spores are also employed in a veterinary vaccine against anthrax. Despite this long lasting and extensive use, the specific contribution of spores to the beneficial effects of probiotics and to the immunogenicity of the vaccine is not completely elucidated. This review focuses on the different aspects of the use of spore preparations. In particular the use of recombinant spores as vaccine delivery vehicles is described and discussed.
OIE (2004). Anthrax. *In* Manual of diagnostic test and vaccines for terrestrial animals. Chapter 2.2.1. Anthrax. http://www.oie.int/eng/normes/mmanual/A_00040.htm	
OIE (2005). Terrestrial animal code. Chapter 2.2.1 Anthrax.	Incubation period 20 days. For ruminants, equines and pigs: International Veterinary Certificate: no signs on day of shipment; were kept for 20 days prior to shipment in an establishment where no case of anthrax was officially declared; were vaccinated not less than 20 days and not more than 6 months prior to shipment.
Okinaka R, Cloud K, Hampton O, Hoffmaster A, Hill K, Keim P, Koehler T, Lamke G, Kumano S, Manter D, Martinez Y, Ricke D, Svensson R, Jackson P. (1999). Sequence, assembly and analysis of pX01 and pX02. J Appl	

Microbiol 87: 261-262	
Okoh AEJ. (1981). An epizootic of anthrax in goats and sheep in Danbatta, Nigeria. Bulletin of Animal Health and Production in Africa.. 29: 4, 355-359.	Between late March and early April 1978, an outbreak of anthrax in three villages in Danbatta, Kano State killed at least 341 goats and 72 sheep during a five week period ending April 1978. Confirmation of the outbreak was based on isolation of *Bacillus anthracis* from specimens collected from the area involved. Strict animal quarantine measures and the use of unencapsulated anthrax spore vaccine were credited with preventing many livestock losses in the area. No human disease was associated with the epizootic. A discussion of the control of anthrax in small ruminants is given. The epizootiology and mode of spread of the disease among goats and sheep are hypothesized.
Okolo MIO (1988). Prevalence of anthrax in emergency slaughtered food animals in Nigeria. Vet Rec 122: 636	34.9% of ermergency slaughtered cattle had B anthracis in their milk.
Okolo MIO. (1983). Studies on anthrax in food animals and persons occupationally exposed to the zoonoses in eastern Nigeria. International Journal of Zoology;12:276-282.	
Olsnes S, Wesche J. (2001). Fighting anthrax with a mutant toxin. Science. 292: 5517, 647-648.	This article discusses the molecular pathogenesis, diseases prevention and some therapeutic approaches in treatment of *Bacillus anthracis* infection in man.
Oncu S, Oncu S, Sakarya S. (2003). Anthrax--an overview. Med Sci Monit. 9(11):RA276-83.	Department of Infectious Doseases and Clinical Microbiology, Faculty of Medicine, Adrian Menderes University, 09100 Aydin, Turkey. sercanoncu@hotmail.com Anthrax, a disease of mammals (including humans), is caused by a spore-forming Gram-positive bacilli called *Bacillus anthracis*. Anthrax is one of the oldest threats to humanity, and remains endemic in animals in many parts of the world. The incidence of anthrax has decreased in developed countries, but it remains a considerable health problem in developing countries. The disease is transmitted to humans by contact with sick animals or their products, such as wool, skin, meat etc. Capsular polypeptide and anthrax toxin are the principal virulence factors of B. anthracis. Anthrax toxin consists of three proteins called protective antigen, edema factor, and lethal factor, each of which is nontoxic but acts synergistically. Human anthrax has three major clinical forms: cutaneous, inhalational, and gastrointestinal. The diagnosis is easily established in cutaneous cases, characterized by black eschar. Severe intoxication and collapse during the course of bronchopneumonia or hemorrhagic enteritis should prompt suspicion of anthrax. Treatment with antibiotics is mandatory. If untreated, anthrax in all forms can lead to septicemia and death. Recently, considerable attention has been focused on the potential for B. anthracis to be used in acts of biological terrorism. The ease of laboratory production and its dissemination via aerosol led to its adoption by terrorists, as shown by recent events in the USA. A good knowledge of anthrax, its epidemiology, pathogenesis, clinical forms and potential as a biological weapon is essential for timely prevention and treatment. This review summarizes the current knowledge on anthrax.
OraVax, Inc. Joins DynPort In Department of Defense Contract To Develop FDA-Licensed Vaccines Against Potential Biological Warfare Agents CAMBRIDGE, Mass., May 04 /PRNewswire/ -- OraVax,	

Inc.	
Orenstein WA, Hinman AR, Bart KJ et al: (1995). Immunization. In Mandell GL, Bennett JE and Dolin R, eds. Principles and practice of infectious diseases, 4th ed. p.2770,	
Oreshkin AS, Gorelov Yu M, Pankratov LD. (1983). Dried, combined vaccine against anthrax and ovine enterotoxaemia caused by Clostridium perfringens type D. Infektsionnye bolezni sel'skokhoz. zhivotnykh.. Novosibirsk, USSR:. 85-88.	Secondary Journal Source: Referativnyi Zhurnal, 82. Veterinariya 1984, No. 8 abstract 8.82.70.
Orfeur NB, Edelstein RM. (1968). Unusual features of an anthrax outbreak. Vet Rec 82: 634-635	"the use of intravenous penicillin in this outbreak shows how effective this method of treatment is when used in the very early stages of the disease."
Oriani DS, Staskevich AS. (2004). Pathogens detected by the bacteriological service of the Faculty of Veterinary Sciences, UNLPam., General Pico, La Pampa, Argentina. (Part I). Revista de Medicina Veterinaria (Buenos Aires).. 85: 4, 166-169.	[Spanish] The laboratory of clinical bacteriology that plays a major role in the detection of agents causing infectious diseases serves as a fundamental tool for the Veterinary Clinician in the treatment and prophylaxis of diseases. The objective of this publication is to show the aetiologies of most frequently occurring infectious diseases of bacterial and/or mycotic source in the area city of General Pico, La Pampa province. A total of 154 samples were processed, 80 belonging to large animals, 63 to small animals and 11 to wild animals. 34% of the samples were collected in 2002 and 66% during 2003. Conventional methods for microbiological diagnosis were used. 11 bacterial genres were isolated from large animals (Staphylococcus sp., Haemophilus sp., Pasteurella sp., Brucella sp., Campylobacter sp., Clostridium sp., Moraxella sp., Streptococcus sp., Actinobacillus sp., Mycobacterium sp. and *Bacillus anthracis*), 4 bacterial and 2 mycotic genres from small animals (Proteus sp., Staphylococcus sp., Pseudomonas sp., Klebsiella sp., Dermatophytos sp. and Malassezia pachydermatis), with Staphylococcus intermedius as the predominant species responsible of canine dermatitis and otitis. Yersinia pseudotuberculosis was isolated in 100% from the granulomatous lesions in European hare.
PAHO (2003). Anthrax. In Zoonoses and communicable diseases common to man and animals, 3rd edition, Pan American Health Organization, WHO, Washington DC, pp 21-28	'In regions where anthrax occurs sporadically, mass vaccination is not justified and should be limited to affected herds. Rapid diagnosis, isolation and treatment of sick animals with antibiotics (penicillin) are important.'
Pani DN, Panda SN. (1994). Comparative studies on different culture media in the production of anthrax spore vaccine. Indian Journal of Animal Health.. 33: 1, 5-8.	
Pankratov LD, Oreshkin AS, Gorelov YuM. (1977). Trials of a combined vaccine against anthrax and clostridial	The safety and immunogenicity of the freeze-dried combined vaccine was tested in laboratory animals and sheep. It consisted of the 'STI' anthrax vaccine, and an alum-precipitated anatoxin against Clostridium perfringens type D toxin. Immuno-genicity in relation to anthrax was tested in guinea-pigs, rabbits and sheep, which resisted challenge with 1-10

enterotoxaemia of sheep. Veterinariya, Moscow.. 2, 38-39.	median lethal doses (LD50)of virulent B. anthracis. Also, the epsilon antitoxin values were determined in vaccinated rabbits and sheep; 58 rabbits and 41 sheep withstood challenge with 1-3 LD50 of Cl. perfringens epsilon toxin. The vaccine was used in thousands of sheep under field conditions, either two or three injections. It was concluded that the vaccine afforded protection for more than a year.
Pannifer AD, Wong TY, Schwarzenbacher R, Renatus M, Petosa C, Bienkowska J, Lacy DB, Collier RJ, Park S, Leppla SH, Hanna P, Liddington RC (2001). Crystal structure of the anthrax lethal factor. Nature 414: 229-233	LF is a protein with relative molecular mass 90,000. It is a highly specific protease that cleaves members of the mitogen-activated protein kinase kinase (MAPKK) family near to the amino termini, leading to the inhibition of one or more signalling pathways. The crystal structure is described as containing four domains. The structure reveals an enzyme with high and unusual specificity.
Park ChaeWoong; Park ChulMin; Lee InJae; Kim KiJeong; Kim WonYong; Chung SangIn; Choi ChulSoon. (2000). Identification of *Bacillus anthracis* by immunofluorescent antibody technique. Chung-Ang Journal of Medicine.. 25: 4, 185-193.	[Korean] Anthrax specific antigens were isolated from cell-associated antigens and their specificity was examined. The antigens reacted specifically to anthrax immune serum using western blotting but did not react to immune serums of closely related species. Molecular weight of the purified antigen was 39 kDa. This antigen was specific only to serum against B. anthracis. Rabbit immune serum against purified antigen reacted strongly to the 39-kDa antigen. Polyclonal antibody against the 39-kDa antigen reacted only with B. anthracis in immunofluorescent antibody test.
Park JM, Greten FR, Li ZW, KarinM (2002). Macrophage apoptosis by anthrax lethal factor through p38 MAP kinase inhibition. Science 297: 2048–51.	[During et al 2001, 12]
Pasteur L, Chamberland, Roux. (2002). Summary report of the experiments conducted at Pouilly-le-Fort, near Melun, on the anthrax vaccination, 1881. Yale J Biol Med. 75(1): 59-62	Publication Types: Biography Classical Article Historical Article
Patra G, Vaissaire J, Weber-Levy M, Doujet C le; Mock M. (1998). Molecular characterization of Bacillus strains involved in outbreaks of anthrax in France in 1997. Journal of Clinical Microbiology.. 36: 11, 3412-3414.	Outbreaks of anthrax occurred in 2 regions of France in 1997. 93 cows and 1 calf died, and there were 3 non-fatal cases in humans. The diagnosis of anthrax was rapidly confirmed by bacteriological and molecular biological methods. The strains of *Bacillus anthracis* in animal and soil samples were identified by a multiplex PCR assay. They all belonged to the variable-number tandem repeat (VNTR) group (VNTR)3. A penicillin-resistant strain was detected. Non-virulent bacilli related to B. anthracis, of all VNTR types, were also found in the soil.
Pellizzari R, Guidi-Rontani C, Vitale G, Mock M, Montecucco C (1999). Anthrax lethal factor cleaves MKK3 in macrophages and inhibits the LPS/ IFNg-induced release of NO and TNFa. FEBS Lett 462: 199–204.	[During et al 2001, 33]
Pepper IL, Gentry TJ. (2002). Incidence of *Bacillus anthracis* in soil. Soil Science.	Interest in anthrax has increased recently due to its use in bioterrorism attacks. *Bacillus anthracis*, the causative agent of anthrax, is genetically similar to other Bacillus spp. that occur in the environment, and is known to persist in soil for

167: 10, 627-635.	years in the form of spores. In many soils, naturally occurring anthrax infections tend to occur during dry periods following a wet period. The "incubator theory" suggests that spores are concentrated in low-lying areas during rainfall events and animals are subsequently exposed to contaminated soil during foraging in dry periods. It is currently believed that B. anthracis spores require a host for germination, and thus do not undergo proliferation cycles in the soil. There is a potential for B. anthracis spores to be transported as an aerosol, but human infection due to inhalation of spores is unlikely given the high minimum infectious dose required to cause disease. Historically, naturally occurring anthrax infections in humans have most commonly occurred due to contact with diseased animals or animal products. However, B. anthracis has been developed as a biological weapon with accidental and intentional releases resulting in human death. With the development of an anthrax vaccine, anthrax outbreaks have generally been controlled in developed countries; however, anthrax is still a major problem in many parts of the world. The advent of molecular techniques has enhanced the detection of B. anthracis spores, which is now possible in less than 1 h. However, due to the persistence of spores, it is difficult to eliminate B. anthracis contamination from the environment. There remains a need for additional research on anthrax in several areas including evaluation of the conditions that favor B. anthracis survival in soil, determination of whether B. anthracis undergoes a growth cycle in soil, and determination of the potential for transfer of B. anthracis virulence genes to other soil microorganisms.
Perdue ML, Karns J, Higgins J, Kessel JA van. (2003). Detection and fate of *Bacillus anthracis* (Sterne) vegetative cells and spores added to bulk tank milk. Journal of Food Protection. 66: 12, 2349-2354.	A preparation of *Bacillus anthracis* (Sterne strain) spores was used to evaluate commercially available reagents and portable equipment for detecting anthrax contamination by using real-time PCR and was used to assess the fate of spores added directly to bulk tank milk. The Ruggedized Advanced Pathogen Identification Device (RAPID) was employed to detect spores in raw milk down to a concentration of 2500 spores per ml. Commercially available primers and probes developed to detect either the protective antigen gene or the lethal factor gene both provided easily read positive signals with the RAPID following extraction from milk with a commercially available DNA extraction kit. Nucleotide sequence analysis of the vrrA gene with the use of DNA extracted from spiked milk provided molecular data that readily identified the spores as B. anthracis with a 100% BLAST match to the Sterne and Ames strains and easily distinguished them from B. cereus. Physical-fate and thermal-stability studies demonstrated that spores and vegetative cells have a strong affinity for the cream fraction of whole milk. A single treatment at standard pasteurization temperatures, while 100% lethal to vegetative cells, had no effect on spore viability even 14 days after the treatment. Twenty-four hours after the first treatment, a second treatment at 72 degrees C for 15 s reduced the viability of the population by ca. 99% but still did not kill all of the spores. From these studies, we conclude that standard pasteurization techniques for milk would have little effect on the viability of B. anthracis spores and that raw or pasteurized milk poses no obstacles to the rapid detection of the spores by molecular techniques.
Perkins BA, Popovic T, Yeskey K. (2002). Public health in the time of bioterrorism. Emerg. Infect. Dis. 8, 1015– 1018.	[Laboratory Response Network for Bioterrorism and the National Pharmaceutical Stockpile]
Perkins SD, Flick-Smith HC, Garmory HS, Essex-Lopresti AE, Stevenson FK, Phillpotts RJ (2005). Evaluation of the VP22 protein for enhancement of a DNA vaccine against anthrax. Genetic Vaccines and Therapy 3:3	Biomedical Sciences Department, Defence Science and Technology Laboratory, Porton Down, Salisbury, Wiltshire, SP4 OJQ, UK, sdperkins@dstl.gov.uk Background: Previously, antigens expressed from DNA vaccines have been fused to the VP22 protein from Herpes Simplex Virus type I in order to improve efficacy. However, the immune enhancing mechanism of VP22 is poorly understood and initial suggestions that VP22 can mediate intercellular spread have been questioned. Despite this, fusion of VP22 to antigens expressed from DNA vaccines has improved immune responses, particularly to non-secreted antigens. Methods: In this study, we fused the gene for the VP22 protein to the gene for Protective Antigen (PA) from *Bacillus anthracis*, the causative agent of anthrax. Protective immunity against infection with B. anthracis is almost

	entirely based on a response to PA and we have generated two constructs, where VP22 is fused to either the N- or the C-terminus of the 63 kDa protease-cleaved fragment of PA (PA63). Results: Following gene gun immunisation of A/J mice with these constructs, we observed no improvement in the anti-PA antibody response generated. Following an intraperitoneal challenge with 70 50% lethal doses of B. anthracis strain STI spores, no difference in protection was evident in groups immunised with the DNA vaccine expressing PA63 and the DNA vaccines expressing fusion proteins of PA63 with VP22. Conclusion: VP22 fusion does not improve the protection of A/J mice against live spore challenge following immunisation of DNA vaccines expressing PA63.
Perreten V, Vorlet-Fawer L, Slickers P, Ehricht R, Kuhnert P, Frey J. (2005). Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. J Clin Microbiol. 43(5):2291-302.	Institute of Veterinary Bacteriology, University of Berne, Langass-Strasse 122, Postfach, CH-3001 Bern, Switzerland. vincent.perreten@vbi.unibe.ch A disposable microarray was developed for detection of up to 90 antibiotic resistance genes in gram-positive bacteria by hybridization. Each antibiotic resistance gene is represented by two specific oligonucleotides chosen from consensus sequences of gene families, except for nine genes for which only one specific oligonucleotide could be developed. A total of 137 oligonucleotides (26 to 33 nucleotides in length with similar physicochemical parameters) were spotted onto the microarray. The microarrays (ArrayTubes) were hybridized with 36 strains carrying specific antibiotic resistance genes that allowed testing of the sensitivity and specificity of 125 oligonucleotides. Among these were well-characterized multidrug-resistant strains of Enterococcus faecalis, Enterococcus faecium, and Lactococcus lactis and an avirulent strain of *Bacillus anthracis* harboring the broad-host-range resistance plasmid pRE25. Analysis of two multidrug-resistant field strains allowed the detection of 12 different antibiotic resistance genes in a Staphylococcus haemolyticus strain isolated from mastitis milk and 6 resistance genes in a Clostridium perfringens strain isolated from a calf. In both cases, the microarray genotyping corresponded to the phenotype of the strains. The ArrayTube platform presents the advantage of rapidly screening bacteria for the presence of antibiotic resistance genes known in gram-positive bacteria. This technology has a large potential for applications in basic research, food safety, and surveillance programs for antimicrobial resistance.
Personeus G, Cooper MS, Percival RC (1956). Studies on an anthrax vaccine prepared from nonencapsulated variants of *Bacillus anthracis*. Am J Vet Res 17: 153-156	Spore-dose response relationship in guinea pigs Sheep: Sterne vaccine with 0.25% saponin, 1ml dose, 20 million spores.
Peterson MJ, Davis DS, Templeton JW. (1993). An enzyme-linked immunosorbent assay for detecting anthrax antibody in white-tailed deer (Odocoileus virginianus): evaluation of anthrax vaccination and sera from free-ranging deer. J Wildl Dis 29(1): 130-5.	Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station 77843-2258. An enzyme-linked immunosorbent assay for anthrax antibody in white-tailed deer (Odocoileus virginianus) was developed and used to evaluate a vaccination study and compare sera from hunter-killed deer in anthrax endemic and non-endemic areas. Deer subcutaneously vaccinated with anthrax avirulent spore vaccine developed specific antibody to protective antigen (PA) which was significantly higher than the non-vaccinated controls at 30, 60, 90, and 240 days post-vaccination. There was no difference between the levels of antibody to PA between deer in anthrax endemic and non-endemic areas.
Petrovszky M, Grasoiu G, Grigoriu N, Danescu A, Faur G. (1971). [Mixed anaerobic enterotoxaemia and anthrax vaccine for sheep]. Lucrarile Institutului de Cercetari Veterinare si Biopreparate	[Romanian] A combined vaccine consisting of 90% Clostridium welchii and 10% *Bacillus anthracis* spore vaccine, and containing 0.009% g% formalin and 1:100,000 sodium merthiolate, could be stored for at least 12 months. Antibody titres induced in sheep and g.pigs were similar to those induced by the component vaccines alone. A 100% immunity against anthrax lasting at least 7 months was induced in sheep.

'Pasteur'.. 7: Part I, 145-154.	
Pezard C, Berche P, Mock M. (1991). Contribution of individual toxin components to virulence of *Bacillus anthracis*. Infection and Immunity.. 59: 10, 3472-3477.	Three proteins, protective antigen (PA), lethal factor (LF), and oedema factor (EF; a calmodulin-dependent adenylate cyclase), compose the lethal (PA + LF) and oedema (PA + EF) toxins secreted by B. anthracis. Mutant strains, each deficient in the production of one toxin component, were constructed, and their virulence was studied. A kanamycin resistance cassette was inserted in each cya (encoding EF) and lef (encoding LF) gene, and the constructs were separately introduced into B. anthracis Sterne on a mobilizable shuttle plasmid. An EF- strain and an LF- strain were then isolated after homologous recombination with the resident toxin-encoding plasmid, pXO1. Spores from these mutants and from a previously constructed PA- mutant were used to inoculate mice, and the lethality and local oedema formation were monitored. LF- or PA- mutants were not lethal even at high inocula, whereas the EF- mutant induced lethal infections. This indicates that LF in combination with PA is a key virulence factor required for lethality. Skin oedema formation was observed with the LF- mutant, which produces only the combination of PA and EF. However, EF- and LF- mutants were less efficient at inducing, respectively, lethality and oedema than was the parental Sterne strain. These results suggest that the 3 toxin components might act synergistically in vivo to cause lethality and oedema formation.
Pezard C, Duflot E, Mock M (1993). Construction of Bacillus anthracis mutant strains producing a single toxin component. J Gen Microbiol 139: 2459-2463	Laboratoire de Genetique Moleculaire des Toxines (URA 557, CNRS), Institut Pasteur, Paris, France. The two protein exotoxins secreted by Bacillus anthracis are composed of three distinct components: protective antigen (PA), lethal factor (LF), and (o)edema factor (EF). We have developed a genetic strategy that permits us selectively to inactivate each of the genes coding for PA, EF or LF. This strategy involved the deletion of a portion of the structural gene and the insertion of an antibiotic resistance cassette. With this technique, double mutant strains of B. anthracis producing only one toxin component have been constructed. Characterization of the mutant strains indicated that they produced the expected single toxin protein. Using a simple, two-step protocol, we have purified PA, LF and EF to homogeneity from culture supernatants. These three mutant strains are potentially powerful tools for studying the individual effect of each toxin component in vitro and in vivo.
Pezard C, Sirard JC, Mock M. (1996). Protective immunity induced by Bacillus anthracis toxin mutant strains. Adv Exp Med Biol 397: 69-72	
Pezard C, Weber M, Sirard JC, Berche P, Mock M. (1995). Protective immunity induced by *Bacillus anthracis* toxin-deficient strains. Infection and Immunity.. 63: 4, 1369-1372.	The 2 toxins secreted by B. anthracis are composed of binary combinations of 3 proteins: protective antigen (PA), lethal factor (LF), and oedema factor (EF). Six mutant strains that are deficient in the production of one or 2 of these toxin components have been previously constructed and characterized (C. Pezard, E. Duflot, and M. Mock, J. Gen. Microbiol. 139:2459-2463, 1993). The antibody response to the in vivo production of PA, LF, and EF was examined in mice immunized with spores of strains producing these proteins. High titres of antibody to PA were observed after immunization with all strains producing PA, while titres of antibodies to EF and LF were weak in animals immunized with strains producing only EF or LF. Immunization with strains producing either PA and EF or PA and LF resulted in an increased antibody response to EF or LF, respectively. The differing levels of protection from a lethal anthrax challenge afforded to mice immunized with spores of the mutant strains not only confirm the role of PA as the major protective antigen in the humoral response but also indicate a significant contribution of LF and EF to immunoprotection. It was shown that PA-deficient strains were also able to provide some protection, thereby suggesting that immune mechanisms other than the humoral response may be involved in immunity to anthrax. A control strain lacking the toxin-encoding plasmid was unable to provide protection or elicit an antibody response against bacterial antigens, indicating a possible role for pXO1 in the survival of B. anthracis in a host.

Phillips AP, Ezzell JW. (1989). Identification of *Bacillus anthracis* by polyclonal antibodies against extracted vegetative cell antigens. Journal of Applied Bacteriology.. 66: 5, 419-432.	Chemical Defence Establishment, Salisbury, Wiltshire, UK. The extractable protein antigens EA1 and EA2 of *Bacillus anthracis* were prepared from electrophoresis transblots of SDS extracts of vegetative bacteria of the Sterne strain. Hyperimmune guinea-pig antiserum against EA2 failed to react with B. anthracis cells in immunofluorescence (IF) tests. Guinea-pig antiserum against EA1 (anti-EA1) reacted strongly in IF tests with non-encapsulated vegetative cell of 10 of 12 strains of B. anthracis and with cells of strains of B. cereus and B. thuringiensis. The unreactive B. anthracis strains were delta-Vollum-1B-1 and Texas. Encapsulated cells of B. anthracis stained poorly except for small bright regions. Absorption of anti-EA1 with cells of B. cereus NCTC 8035 and NCTC 9946 removed activity towards all B. cereus strains tested, but only partly reduced cross-reaction with B. thuringiensis strains. Absorption of anti-EA1 with B. thuringiensis 4041 removed activity towards this strain and B. cereus strains. Evidence is produced that B. thuringiensis cells grown on nutrient agar possess more cross-reacting antigens than cells grown in nutrient broth. The reaction of anti-EA1 with Bacillus spores immobilized in clumps on microscope slides was attributed to contaminating vegetative debris because well-separated individual spores failed to react. A rapid IF test was developed allowing identification of B. anthracis sampled from overnight cultures on blood plates. When sodium dodecyl sulphate extracts of B. anthracis vegetative cells were analysed on immunoblots (Western blots) by reaction with anti-EA1, a number of bands were visualized in addition to the expected 91 kiloDalton EA1 band. Prior absorption of anti-EA1 with B. cereus or B. thuringiensis cells resulted in the disappearance of most or all of the brands in blots of these species, but had less effect on blots of the B. anthracis strains. All six B. anthracis strains that were blotted including delta-Vollum-1B-1 and Texas, could thus be distinguished from B. cereus and B. thuringiensis by their differential reaction with unabsorbed and absorbed anti-EA1.
Phillips AP, Martin KL, Broster MG. (1983). Differentiation between spores of *Bacillus anthracis* and Bacillus cereus by a quantitative immunofluorescence technique. Journal of Clinical Microbiology.. 17: 1, 41-47.	
Phillips AP, Martin KL, Cross NL, Drake RG. (1984). Evaluation of immunoradiometric and ELISA versions of a microtitre plate assay for *Bacillus anthracis* spores. Journal of Immunological Methods.. 70: 1, 75-81.	Solid-phase indirect labelled antibody assays for B. anthracis spores heat fixed on polystyrene microtitre plates were compared as immunoradiometric assay (IRMA) and enzyme-linked immunosorbent assay (ELISA) versions. Signal-to-noise ratios were usually higher in the IRMA than in the ELISA performed under parallel conditions but replicates were more varied in the IRMA. The antigen detection threshold and resolution limit calculated after regression analysis were broadly comparable in the two assays.
Phillips AP, Martin KL. (1983). Comparison of direct and indirect immunoradiometric assays (IRMA) for *Bacillus anthracis* spores immobilised on multispot microscope slides. Journal of Applied Bacteriology.. 55: 2, 315-324.	A solid-phase immunoradiometric assay (IRMA) is described in which *Bacillus anthracis* spores were heat fixed to the wells of glass multispot microscope slides. Assays for spores of B. anthracis Vollum and Sterne strains with superscript 3H labels were evaluated in the direct and indirect versions. Although the indirect method tended to be more sensitive than the direct method (higher signal-to-noise ratio), the elevated ratio was shown to correspond to high debris content in the spore preparation. Another disadvantage of the indirect method was a prozone effect. For the differentiation of B. anthracis spores from B. cereus spores immunopurified antibody prepared from IgG gave least cross-reactions, but sensitivity was not increased, and the authors consider that there may be a better way to produce an antibody reagent for the test such as monoclonal antibodies. The importance of using spore preparations purified by Percoll for raising antisera is stressed.
Phillips AP, Martin KL. (1983). Comparison	

of immunoradiometric assays of *Bacillus anthracis* spores immobilised on multispot slides and on microtitre plates. Journal of Immunological Methods.. 62: 3, 273-282.	
Phillips AP, Martin KL. (1988). Investigation of spore surface antigens in the genus Bacillus by the use of polyclonal antibodies in immunofluorescence tests. Journal of Applied Bacteriology.. 64: 1, 47-55.	Fluorescein-conjugated rabbit antibodies to formalized spores of *Bacillus anthracis* were tested against strains of B. anthracis and other Bacillus species in a subjective immunofluorescence test. The lack of reaction of B. anthracis Vollum spores with conjugated antibody raised against B. anthracis Sterne spores indicated that spores of the Vollum strain lacked a major surface antigen present in most of the other anthrax strains tested, including the non-encapsulated strains Sterne and the Soviet ST1, variants cured of the pX01 plasmid that codes for the toxin, and several virulent strains. Four other antibody preparations, raised against B. anthracis Vollum, New Hampshire, Ames and Strain 15, reacted to an approximately similar degree with spores of all four strains and of Sterne, indicating that Vollum has at least one spore antigen in common with these other strains. The anti-Sterne and anti-Vollum conjugates both displayed cross-reactions with spores of strains of B. cereus, B. coagulans, B. subtilis, B. megaterium, B. polymyxa, B. pumilus and B. thuringiensis. Absorption of the anti-anthrax conjugates with B. cereus NCTC 8035 and NCTC 10320 removed all these cross-reactions, demonstrating the existence of spore antigens specific for anthrax.
Phipps AJ, Premanandan C, Barnewall RE, Lairmore MD. (2004). Rabbit and nonhuman primate models of toxin-targeting human anthrax vaccines. Microbiology and Molecular Biology Reviews. . 68: 4, 617-629.	The intentional use of *Bacillus anthracis*, the etiological agent of anthrax, as a bioterrorist weapon in late 2001 made our society acutely aware of the importance of developing, testing, and stockpiling adequate countermeasures against biological attacks. Biodefense vaccines are an important component of our arsenal to be used during a biological attack. However, most of the agents considered significant threats either have been eradicated or rarely infect humans alive today. As such, vaccine efficacy cannot be determined in human clinical trials but must be extrapolated from experimental animal models. This article reviews the efficacy and immunogenicity of human anthrax vaccines in well-defined animal models and the progress toward developing a rugged immunologic correlate of protection. The ongoing evaluation of human anthrax vaccines will be dependent on animal efficacy data in the absence of human efficacy data for licensure by the U.S. Food and Drug Administration. 'If a single immune parameter, such as anti-PA IgG titre, can be identified as the primary surrogate marker of protection, logistic regression analysis can be applied to determine the effect of titer on survival outcome. … If multiple immune parameters are identified as criticial surrogate markers of protection, predicitive models may be built by using several approaches, including logistic discriminant analysis and cluster analysis.'
Pienaar U de V. (1967). Epidemiology of anthrax in wild animals and the control of anthrax epizootics in the Kruger National Park, South Africa. Federation Proceedings 26: 1496-1501	Three epizootics in Kruger NP described. Watering points were important sources of infection. Carnivorous birds, carrion eaters and insects were instrumental in spreading disease. Fire was successfully used to destroy some sources of infection. Some success in treating infected water with quaternary ammonium compounds. Protection against recurrence only possible through immunization. "a concentrated nonliving vaccine would seem to be indicated in this event because in some game animals the use of even a very attenuated live vaccine is not safe."
Pitt ML, Ivins BE, Estep JE et al: (1996) Comparison of the efficacy of purified protective antigen and MDPH to protect non-human primates from inhalation anthrax. Salisbury Medical Bulletin,	

special supplement no. 87. June 1996, p130.	
Pitt ML, Little S, Ivins BE, Fellows P, Boles J, Barth J, Hewetson J, Friedlander AM. (1999). In vitro correlate of immunity in an animal model of inhalational anthrax J Appl Microbiol. 87(2):304.	The incidence of anthrax in humans is extremely low. Human vaccine efficacy studies for inhalational anthrax cannot be conducted. The identification of a correlate of protection that predicts vaccine efficacy is crucial for determining the immune status of immunized humans. This surrogate marker of immunity can only be established by using an appropriate animal model. Numerous studies showed that protective antigen (PA) is the principle protective antigen in naturally- or vaccine-induced immunity. However, attempts to correlate the quantity of anti-PA antibodies with protective immunity in the guinea pig model for anthrax and various vaccine formulations have failed. In these studies, we used the licensed anthrax vaccine adsorbed (AVA) in rabbits. Groups of New Zealand white rabbits, 10 or 20 per group, were immunized intramuscularly (two doses, 4 weeks apart) with varying doses of AVA, ranging from a human dose to 1:256 dilution in sterile phosphate-buffered saline (PBS). Control rabbits received PBS/Alhydrogel according to the same schedule. Each rabbit was bled 2 weeks after the second dose, and antibody levels to PA measured by both the quantitative anti-PA IgG ELISA and the toxin-neutralizing antibody (TNA) assay. Rabbits were aerosol-challenged 10 weeks from day 0 with a lethal dose of Ames spores. All the rabbits that received the undiluted and 1:4 dilution of vaccine survived, whereas those receiving the higher dilutions of vaccine (1:16, 1:64 and 1:256) had deaths in their groups. All the controls died. Rabbit survival was compared with the antibody response. Statistical models were used to test for significance of the peak antibody responses to predict survival. Results showed that both the amount of anti-PA IgG and TNA titres present in the sera at the time of the peak antibody response were significant ($P < 0.0001$) predictors of survival. These results demonstrate that the humoral immune response to AVA can predict protection in the rabbit model of inhalational anthrax.
Pitt ML, Little SF, Ivins BE, Fellows P, Barth J, Hewetson J, Gibbs P, Dertzbaugh M, Friedlander AM. (2001). In vitro correlate of immunity in a rabbit model of inhalational anthrax. Vaccine. 19(32):4768-73.	United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011, USA. louise.pitt@amedd.army.mil A serological correlate of vaccine-induced immunity was identified in the rabbit model of inhalational anthrax. Animals were inoculated intramuscularly at 0 and 4 weeks with varying doses of Anthrax Vaccine Adsorbed (AVA) ranging from a human dose to a 1:256 dilution in phosphate-buffered saline (PBS). At 6 and 10 weeks, both the quantitative anti-protective antigen (PA) IgG ELISA and the toxin-neutralizing antibody (TNA) assays were used to measure antibody levels to PA. Rabbits were aerosol-challenged at 10 weeks with a lethal dose (84-133 LD(50)) of *Bacillus anthracis* spores. All the rabbits that received the undiluted and 1:4 dilution of vaccine survived, whereas those receiving the higher dilutions of vaccine (1:16, 1:64 and 1:256) had deaths in their groups. Results showed that antibody levels to PA at both 6 and 10 weeks were significant ($P<0.0001$) predictors of survival.
Pittman PR (2000). Comparative study to determine the best two dose schedule and route of administration of human anthrax vaccine. Final Study Report to the FDA, Fort Detrick MD, US Army Medical Research Institute of Infectious Diseases	[Brachman et al 2004, 126]
Pittman PR, Gibbs PH, Cannon TL, Friedlander AM. (2001). Anthrax vaccine: short-term safety experience in humans. Vaccine. 20(5-6): 972-978.	Division of Medicine, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702-5011, USA. phillip.pittman@det.amedd.army.mil *Bacillus anthracis* is the major terrorist and biological warfare agent of concern to civilian and military medical planners. The licensed anthrax vaccine, adsorbed (AVA) is believed to be an effective prophylactic medical countermeasure

	against this threat. Our objective in this report was to expand the safety database for this vaccine by assessing data on self-reported, short-term safety of AVA during more than 25 years of use, measured by local and systemic adverse events temporally associated with the administration of AVA. A minority of AVA recipients reported systemic and injection site reactions. Females reported a higher incidence of injection site and systemic adverse events than males. Data show a difference in incidence of local reactions between lots. A prospective, randomized, placebo-controlled study to actively examine reactogenicity is needed to more completely define the extent and nature of reactions associated with receipt of AVA in humans as well as to confirm the gender lot differences in local reaction rates.
Pittman PR, Hack D, Mangiafico J, Gibbs P, McKee KT Jr, Friedlander AM, Sjogren MH. (2002). Antibody response to a delayed booster dose of anthrax vaccine and botulinum toxoid. Vaccine. 20(16): 2107-2115	United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, USA. philip.pitmann@det.amedd.army.mil We evaluated the prevalence and concentration of serum antibodies 18-24 months after primary inoculation with anthrax and botulinum vaccines, and assessed the reactogenicity and immunogenicity of a significantly delayed booster dose of these vaccines. Five hundred and eight male active-duty military personnel received one, two or three inoculations with anthrax vaccine and/or botulinum toxoid in 1990/1991 in preparation for Operations Desert Shield/Desert Storm. Subjects were vaccinated with the licensed anthrax vaccine, adsorbed (AVA) and pentavalent (ABCDE) botulinum toxoid (PBT) BB-IND 3723. Anthrax protective antigen (PA) IgG antibody was measured in serum using an immunocapture enzyme-linked immunosorbent assay (ELISA). A mouse neutralization test was used to determine the titer of Clostridium botulinum type A antitoxin in serum samples. The prevalence of anti-PA IgG was 30% in individuals 18-24 months after priming with one, two or three doses of AVA. After boosting, 99% of volunteers had detectable anti-PA IgG; only two individuals failed to respond. The prevalence of antibodies against botulinum toxin type A was 28% 18-24 months after initial priming. Following boosting, 99% of volunteers had serum titers >0.02IU/ml, and 97% responded with titers > or =0.25IU/ml.Systemic reactions to booster vaccinations could not be specifically ascribed to one or the other vaccine, but were generally mild and of brief duration. Forty-five percent of volunteers reported one or more systemic reactions over the course of 7 days. Injection site reactions of any kind occurred in 25% of AVA recipients and in 16% of PBT recipients; persistence of local reactions beyond 7 days was infrequent. While the kinetics and durability of immune responses must be studied, these findings suggest that booster doses of anthrax vaccine and botulinum toxoid sufficient to stimulate a robust anamnestic response may be given at times distant from receipt of the primary inoculations.
Pittman PR, Kim-Ahn G, Pifat DY, Coonan K, Gibbs P, Little S, Pace-Templeton JG, Myers R, Parker GW, Friedlander AM. (2002). Anthrax vaccine: immunogenicity and safety of a dose-reduction, route-change comparison study in humans. Vaccine. 20(9-10): 1412-1420	Division of Medicine, United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, MD 21702-5011, USA. phillip.pittman@det.amedd.army.mil Anthrax vaccine adsorbed (AVA), an effective countermeasure against anthrax, is administered as six subcutaneous (SQ) doses over 18 months. To optimize the vaccination schedule and route of administration, we performed a prospective pilot study comparing the use of fewer AVA doses administered intramuscularly (IM) or SQ with the current schedule and route. We enrolled 173 volunteers, randomized to seven groups, who were given AVA once IM or SQ; two doses, 2 or 4 weeks apart, IM or SQ; or six doses at 0, 2, 4 weeks and 6, 12, and 18 months (control group, licensed schedule and route). IM administration of AVA was associated with fewer injection site reactions than SQ administration. Following the first SQ dose of AVA, compared to males, females had a significantly higher rate of injection site reactions such as erythema, induration and subcutaneous nodules (P<0.001). Reaction rates decreased with a longer dose interval between the first two doses. The peak anti-PA IgG antibody response of subjects given two doses of AVA 4 weeks apart IM or SQ was comparable to that seen among subjects who received three doses of AVA at 2-week intervals. The IM route of administering this aluminum hydroxide adsorbed vaccine is safe and has comparable peak anti-PA IgG antibody levels when two doses are administered 4 weeks apart compared to the

	licensed initial dose schedule of three doses administered 2 weeks apart. A large pivotal study is being planned by the Centers for Disease Control and Prevention to confirm these results.
Pittman PR, Mangiafico JA, Rossi CA, Cannon TL, Gibbs PH, Parker GW, Friedlander AM. (2000). Anthrax vaccine: increasing intervals between the first two doses enhances antibody response in humans. Vaccine. 19(2-3): 213-216	Division of Medicine, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011, USA. The influence of dosing interval on the human antibody response to anthrax vaccine adsorbed (AVA) was evaluated in two retrospective serological studies. In both studies, the interval between the first two doses was 2, 3 or 4 weeks. In the first study, banked sera were selected from 89 at-risk individuals at a mean time of 13 days after the second dose of vaccine. In the second study, banked sera were selected from 51 at-risk individuals at a mean time of 48 days following the first dose of AVA. In both studies, the geometric mean anti-protective antigen IgG antibody titer increased significantly as the interval between the two doses increased from 2 to 4 weeks (p=0.0005-0.029). In the first study, the seroconversion rate also increased as the interval between the first two doses increased (p=0. 0034). A prospective, randomized study has been completed and is being analyzed to confirm these findings.
Plotkin SA, Orenstein WA. (1999). Vaccines. Vaccines. W.B. Saunders, Philadelphia, USA:. Ed.3, xix + 1230.	This textbook covers current vaccines against the following diseases: tuberculosis; diphtheria, hepatitis B, Haemophilus influenzae, measles, mumps, pertussis, polio, rubella, tetanus, varicella, streptococcus pneumoniae, adenovirus, anthrax, cholera, hepatitis A, Japanese encephalitis, Neisseria meningitidis, plague, rabies, tick-borne encephalitis, typhoid, and yellow fever. It also covers vaccines of the future including vaccines against cytomegalovirus, influenza, HIV, Lyme disease, parasitic diseases, and rotavirus. The book also discusses the history and general immunology of vaccines and presents an overview of vaccine production. A section is also devoted to public health and regulatory issues relating to vaccination.
Polyakov AA, Kulikovskii AV, Pilipenko VN. (1976). Submicroscopic structure of *Bacillus anthracis* spores subjected to the action of methyl bromide. Doklady Vsesoyuznoi Akademii Sel'skokhozyaistvennykh Nauk.. 12, 23-24.	Sporulated cultures of a vaccine strain of *Bacillus anthracis* were gassed with methyl bromide for varying lengths of time. At 250 g/m3 of the gas, destruction of the spores ranged from 10% after 25 min. exposure, to 99% after 10 h. Exposure to a massive dose (1000 g/m3) for 24-96 h killed all spores. Electron microscope study of the gassed spores showed erosions of the spore walls after the short (25 min.) exposure to the gas. After 10 h, disturbances of the electron density of the cortex and sporoplasm were visible. The 1000 g/m3 dose acted as a fixative of the spores, without causing visible structural change. It is suggested that the methyl bromide acts by alkylating NH2, OH, SH or COOH groups of the proteins or enzymes of the spores.
Polyakov AA, Pilipenko VN, Volkovskii GD, Kulikovskii AV. (1980). Sporicidal activity of methyl bromide and its use as a disinfectant (against spores of *Bacillus anthracis* and other bacilli on wool and hair). Doklady Vsesoyuznoi Akademii Sel'skokhozyaistvennykh Nauk.. 12, 27-29.	Bales of sheep wool or goat hair were experimentally infected with a vaccine strain of *Bacillus anthracis*, B. anthracoides or B. cereus, then fumigated, under plastic cover, with methyl bromide. It was found that all the test bacteria were destroyed in sheep wool bales by exposure for ten days to 4 kg/m3 of methyl bromide, or in goat hair bales by exposure to 3 kg/m3 for 15 days.
Polydorou K. (1983). Cyprus. The campaign against anthrax. World Animal Review.. 46, 41-45.	
Pombo M, Berthold I, Gingrich E, Jaramillo M, Leef M, Sirota L, Hsu H, Arciniega J. (2004). Validation of an anti-PA-ELISA for the potency testing of anthrax vaccine	Center for Biologics Evaluation & Research, US FDA, USA. The potency test for the anthrax vaccine currently licensed for human use in the United States (Anthrax Vaccine Adsorbed) involves the protection of actively immunized guinea pigs from a lethal challenge with a virulent strain of *Bacillus anthracis*. Lethal challenge tests entail the use of specialized containment facilities for the safe and secure

in mice. Biologicals. 32(3):157-63.	handling of the challenge strain. This potential difficulty, plus humane considerations, have prompted us to investigate non-lethal, alternative immunogenicity assays that could be considered as potency tests not only for the current vaccine, but also for vaccines under development. Immunogenicity tests will require suitable measurement of an antibody response to relevant antigens, by methods such as enzyme linked immunosorbent assay (ELISA) or a toxin neutralization assay. Any assay chosen for this purpose should be adequately validated and reproducible by other laboratories. Validation of an analytical procedure requires the demonstration that the assay is suitable for its intended purpose. The objective of this work was to study the performance of an anti-PA-ELISA designed to assess the antibody response to anthrax vaccines in mice. Validation studies were performed according to the guidelines of the International Conference of Harmonization (ICH), and we have established the working range of the assay (37-1159 EU/mL) on the bases of the following parameters: linearity (20-1159 EU/mL; r2=0.99; p-value=0.21), accuracy (91-118% recovery), precision (< or =20%CV, repeatability; < or =9 and < or =21%CV, intermediate precision per day and per analyst, respectively), detection limit (5 EU/mL), and quantification limit (37 EU/mL). We believe that assay specificity and the above characteristics are adequate to allow this ELISA to be considered for use in a mouse immunogenicity (potency) test of anthrax vaccines, and for the standardization of reagents.
Pomerantsev AP, Mockov YUV, Marinin LI et al: (1996). Anthrax prophylaxis by antibiotic resistant strain STI -- AR in combination with urgent antibiotic therapy. Salisbury Medical Bulletin, special supplement #87, June, p 131	
Pomerantsev AP, Shishkova NA, Doronin IP, Sukovatova LV, Marinin LI. (1993). Interaction of *Bacillus anthracis* with benzylpenicillin in vivo and in vitro. Antibiot Khimioter. 38(7):30-3.	Interaction of the cells of *Bacillus anthracis* strain CH-7 with benzylpenicillin was studied. The cells of strain CH-7 were shown to contain the penicillinase gene in the repressed state. Spontaneous derepression of the gene at a rate of 10(-8) resulting in the synthesis of penicillinase was observed. Penicillinase was synthesized constitutionally and its synthesis did not depend on the presence of benzylpenicillin in the cultivation medium. The therapeutic effect of benzylpenicillin in the treatment of the experimental infection induced by the B. anthracis strain producing penicillinase was estimated. The efficacy was shown to depend on the time of the beginning of the antibiotic therapy. When the clinical signs of the infection were evident in the animals contaminated with the penicillinase-producing strain of B. anthracis, their treatment with the mean daily doses of benzylpenicillin failed.
Pomerantsev AP, Staritsin NA, Mockov YV, Marinin LI. (1997). Expression of cereolysine AB genes in *Bacillus anthracis* vaccine strain ensures protection against experimental hemolytic anthrax infection. Vaccine.. 15: 17/18, 1846-1850.	The cereolysin AB genes fYom Bacillus cereus VKM-B164 have been expressed in Bacillus anthracis strains: virulent H-7 (PXOI, PXO2), vaccine STI-1 (PXOI), 221 (without its own plasmids). Expression was achieved by cloning the genes in a high copy number plasmid pE194. This construct was integrated with host genomes in amplified form. Gold hamsters were vaccinated with parental and recombinant B. anthracis STI-1 and 221 strains and challenged with virulent ones subcutaneously, Gold hamsters vaccinated with 221 strains showed absence of protection. STI-1 immunisation protected against the H-7 strain, but did not protect against the recombinant strain. STI-1 recombinant strain protected gold hamsters against the H-7 as well as the recombinant H-7 strains. The results describe the modulation of immunopathogenie properties of B. anthracis due to expression of cereolysin AB genes.
Popov SG, Popova TG, Hopkins S,	Secreted proteolytic enzymes are important pathogenic factors of B anthracis and can be effective therapeutic targets.

Weinstein RS, MacAfee R, Fryxell KJ, Chandhoke V, Bailey C, Alibek K. (2005). Effective antiprotease-antibiotic treatment of experimental anthrax. BMC Infectious Diseases 5: 25	B anthracis culture supernatant contains a number of proteolytic virulence factors with haemorrhagic, caseinolytic and gelatinolytic activity and are distinct from lethal factor. Immune sera raised against these factors as well as chemical inhibitors (phosphoramidon, phenanthroline) provided substantial protective efficacy in combination with antibiotic therapy.
Price BM, Liner AL, Park S (2001). Protection against anthrax lethal toxin challenge by genetic immunization with a plasmid encoding the lethal factor protein. Infect Immun 69: 4509-4515	161
Price LB, Vogler A, Pearson T, Busch JD, Schupp JM, Keim P. (2003). In vitro selection and characterization of *Bacillus anthracis* mutants with high-level resistance to ciprofloxacin. Antimicrob Agents Chemother. 47(7):2362-5.	Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011-5640, USA. Mutants of attenuated *Bacillus anthracis* with high-level ciprofloxacin resistance were isolated using a three-step in vitro selection. Ciprofloxacin MICs were 0.5 micro g/ml for first-step mutants, which had one of two gyrA quinolone resistance-determining region (QRDR) mutations. Ciprofloxacin MICs were 8 and 16 microg/ml for second-step mutants, which had one of three parC QRDR mutations. Ciprofloxacin MICs for third-step mutants were 32 and 64 microg/ml. Mutants for which MICs were 64 microg/ml had one of two additional mutations within the gyrA QRDR or one of two mutations within the gyrB QRDR. Mutants for which MICs were 32 microg/ml had no additional target modifications but showed evidence of enhanced ciprofloxacin efflux.
Proft T. (2005). Microbial toxins: molecular and cellular biology. Horizon Bioscience, Wymondham, UK:. ix + 568.	This multiauthor book provides comprehensive and current knowledge of the molecular and cellular biology of important microbial toxins. The book comprises 20 chapters. The topics covered are: introduction to bacterial protein toxins; activation of a secondary messenger pathways by adenosine diphosphate-ribosylation; microbial toxins that modulate the actin cytoskeleton; cytolethal distending toxin; toxins that interfere with protein synthesis; cell damaging/pore-forming toxins; clostridial neurotoxins; superantigens as microbial toxins that target the immune system; role of toxins in invasive streptococcal disease; toxins in Staphylococcus aureus pathogenesis; the role of anthrax toxins in pathogenesis of *Bacillus anthracis*; clostridial gas gangrene; contribution of Shiga toxin (verotoxin) to the pathogenesis of Shigella and Escherichia coli; the role of Yersinia Ysc-Yop system in pathogenesis; bacteriocins associated with cytotoxicity for eukaryotic cells; mycotoxins; host cell penetration and trafficking of protein toxins; regulation of bacterial toxin expression; modification and improvement of live bacterial vaccines by the use of bacterial cytolysins; microbial toxins as potential tools in bioterrorism. Students, research workers, medical microbiologists, cell biologists, biochemists, and clinicians will find this book very useful.
Prokupek K, Dvorak R, Polacek R. (1979). Anthrax protective antigen and its preparation. Veterinarni Medicina.. 24: 4, 217-224.	[Czech]
Prokupek K, Dvorak R, Polacek R. (1981). Immunogenic effect of anthrax protective antigen. Veterinarni Medicina.. 26: 5, 279-290.	[Czech] A protective antigen (PA) adsorbed on aluminium hydroxide gel, prepared in Czechoslovakia, was used in sheep and goats, subsequently challenged with virulent *Bacillus anthracis*. A single vaccination gave only 60% protection, but a repeated injection, preferably at 14 d interval, gave 100% protection. Peak antibody titre (1:80 to 1:160) was reached two months after vaccination, returning to the initial value after about two more months.
Proskurina VA, Buravtseva NP. (1985). Comparative evaluation of methods for	Comparison of the results of determination of antibiotic sensitivity of the cultures of the anthrax causative agent with the methods of serial dilutions and paper disks revealed coincidence of their antibiotic sensitivity levels. The Unimicon-s

determining the sensitivity of the anthrax microbe to antibiotics. Antibiot Med Biotekhnol. 30(11):845-7.	and AGV media can be used for determination of antibiotic sensitivity of the anthrax causative agent by the above methods.
Provost A, Perreau P. (1978). Combined vaccines for developing countries. Developments in Biological Standardization. 41: 349-360.	[French] Useful combinations were rinderpest + contagious bovine pleuropneumonia (+ anthrax); anthrax + blackleg or sheep pox; pleuropneumonia + blackleg; Newcastle disease + fowl pox + fowl typhoid; avian pasteurellosis + fowl typhoid.
Pullar EM. (1950). The wild (feral) pigs of Australia and their role in the spread of infectious diseases. Aust Vet J 26: 99-110	"The spread of infection in cattle continued until about 500 feral pigs were destroyed by shooting parties."
Puziss M, Manning LC, Lynch JW, Barclay E, Abelow J, Wright GG (1963). Large scale production of protective antigen of *Bacillus anthracis* in anaerobic cultures. Appl Microbiol 11: 330-334	[US vaccine]
Puziss M, Wright GG. (1963). Studies on immunity in anthrax. X. Gel-adsorbed protective antigen for immunization of man. J Bacteriol 85: 230-236	[US Vaccine]
Qi Y, Patra G, Liang X, Williams LE, RoseS, Redkar RJ, et al. (2001). Utilization of the rpoB gene as a specific chromosomal marker for real-time PCR detection of *Bacillus anthracis*. Appl Environ Microbiol;67:3720–7	[Hoffmaster et al 2002]
Quinn CP, SemenovaVA, Elie CM, Romero-Steiner S, Greene C, Li H, Stamey K, Steward-Clark E, Schmidt DS, Mothershed E, Pruckler J, Schwartz S, Benson RF, Helsel LO, Holder PF, Johnson SE, Kellum M, Messmer T, Thacker WL, Besser L, Plikaytis BD, Taylor TH, Freeman AE, Wallace KJ, Dull P, Sejvar J, Bruce E, Moreno R, Schuchat A, Lingappa JR, Marano N, Martin SK, Walls J, Bronsdon M, Carlone GM, Bajani-Ari M, Ashford DA, Stephens DS, Perkins BA (2002). Specific, Sensitive, and Quantitative Enzyme-	The bioterrorism-associated human anthrax epidemic in the fall of 2001 highlighted the need for a sensitive, reproducible, and specific laboratory test for the confirmatory diagnosis of human anthrax. The Centers for Disease Control and Prevention developed, optimized, and rapidly qualified an enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G (IgG) antibodies to *Bacillus anthracis* protective antigen (PA) in human serum. The qualified ELISA had a minimum detection limit of 0.06 µg/mL, a reliable lower limit of detection of 0.09 µg/mL, and a lower limit of quantification in undiluted serum specimens of 3.0 µg/mL anti-PA IgG. The diagnostic sensitivity of the assay was 97.8%, and the diagnostic specificity was 97.6%. A competitive inhibition anti-PA IgG ELISA was also developed to enhance diagnostic specificity to 100%. The anti-PA ELISAs proved valuable for the confirmation of cases of cutaneous and inhalational anthrax and evaluation of patients in whom the diagnosis of anthrax was being considered.

Linked Immunosorbent Assay for Human Immunoglobulin G Antibodies to Anthrax Toxin Protective Antigen. Emerg Infect Dis 8: 1103-1110	
Quinn CP, Shone CC, Turnbull PC, Melling J (1988). Purification of anthrax toxin components by high-performance anion-exchange, gel-filtration and hydrophobic-interaction chromatography. Biochem J 252: 753–8.	[During et al 2001, 16]
Radostits OM, Gay CC, Blood DC, Hinchcliff KW (2000). Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 9th Edition, WB Saunders Company Ltd, London, pages 747-751	
Rainey GJA, Wigelsworth DJ, Ryan PL, Scobie HM, Collier RJ, Young JAT (2005). Receptor-specific requirements for anthrax toxin delivery into cells. PNAS 102: 13278-13283	Infectious Disease Laboratory, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037 The three proteins that constitute anthrax toxin self-assemble into toxic complexes after one of these proteins, protective antigen (PA), binds to tumor endothelial marker 8 (TEM8) or capillary morphogenesis protein 2 (CMG2) cellular receptors. The toxin receptor complexes are internalized, and acidic endosomal pH triggers pore formation by PA and translocation of the catalytic subunits into the cytosol. In this study we show that the pH threshold for conversion of the PA prepore to the pore and for translocation differs by approximately a pH unit, depending on whether the TEM8 or CMG2 receptor is used. For TEM8-associated toxin, these events can occur at close to neutral pH values, and they show relatively low sensitivity to ammonium chloride treatment in cells. In contrast, with CMG2-associated toxin, these events require more acidic conditions and are highly sensitive to ammonium chloride. We show, furthermore, that PA dissociates from TEM8 and CMG2 upon pore formation. Our results are consistent with a model in which translocation depends on pore formation and pore formation, in turn, depends on release of PA from its receptor. We propose that because PA binds to CMG2 with much higher affinity than it does to TEM8, a lower pH is needed to attenuate CMG2 binding to allow pore formation. Our results suggest that toxin can form pores at different points in the endocytic pathway, depending on which receptor is used for entry.
Ramisse V, Patra G, Garrigue H, Guesdon GL, Mock M. (1996). Identification and characterization of *Bacillus anthracis* by multiplex PCR analysis of sequences on plasmids pXO1 and pXO2 and chromosomal DNA. FEMS Microbiol Lett 145: 9–16.	[Hoffmaster et al 2002]
Ramisse V, Patra G, Vaissaire J, Mock M.	

(1999). The Ba813 chromosomal DNA sequence effectively traces the whole *Bacillus anthracis* community. J Appl Microbiol 87: 224-228	
Rao VP, Choudhuri PC, Rao MVS. (1994). Purification and characterization of protective antigen (PA) of B. anthracis. Indian Veterinary Journal.. 71: 11, 1067-1069.	Crude protective antigen of *Bacillus anthracis* was purified by the trichloroacetic acid method and then subjected to PAGE and Sephadex G25 column chromatography. The average yield of purified PA was 1.2 micro g/ml. The results were comparable with those obtained for reference PA. The molecular weight of the purified PA was estimated to be within the range of 35 to 71 kDa.
Rao VP, Choudhuri PC. (1992). Production of protective antigen of B. anthracis in chemically defined medium. Indian Veterinary Journal.. 69: 2, 99-102.	Casamino acid medium, modified Casamino acid medium and a chemically defined 'R' medium were inoculated with 2 x 106 spores of Sterne's strain of *Bacillus anthracis*. Luxuriant growth followed after 48-72 h, and spore counts ranging from 9 x 109-1.3 x 1010/ml of stock suspension were recorded, using optical density measurements and Brown's opacity tubes. The supernatant of the culture flasks was decanted, centrifuged and filtered through sintered glass filters. The filtrate contained only the protective antigen (PA) fraction of the toxin. This fraction is more immunogenic than other toxin components (oedema factor and lethal factor) and its use in vaccine preparation is discussed. By protection and challenge tests in rabbits and guinea pigs the PA obtained was shown to be comparable with reference PA.
Rao VP, Choudhuri PC. (1993). Immunological characterisation of protective antigen (PA) of *Bacillus anthracis*. Indian Veterinary Journal.. 70: 8, 691-694.	Protective antigen (PA), the most immunogenic of the 3 toxic factors produced by B. anthracis, was characterized by the leukocyte migration inhibition test (LMIT), indirect haemagglutination (IHA), intradermal tests and challenge inoculation in guineapigs and rabbits. The optimum concentration of PA, that gave 80-87% inhibition of migration in the LMIT, was 90-180 micro g/ml. The active humoral immunity produced was directly proportional to the purity of the PA preparations. In passive immunity trials there was not full protection from challenge, although death was delayed, compared to controls. Multiple doses of PA, with or without Freund's complete adjuvant or P. multocida vaccine as adjuvant produced stronger active immunity than single doses in challenge trials.
Regan JC: (1944). The local and general serum treatment of cutaneous anthrax. JAMA 77:, 1921	
Rengel J, Bohnel H. (1994). Preliminary studies on oral immunization of wildlife against anthrax. Berliner und Munchener Tierarztliche Wochenschrift.. 107: 5, 145-149.	As a pilot trial for the vaccination of game in African game parks against anthrax, trials with guineapigs were undertaken to vaccinate the animals orally against anthrax. The vaccine was prepared with the Goettingen Bioreactor Technology in which sporulation is reached in the suspension. Guineapigs vaccinated orally or s.c. with the vaccine resisted a challenge of 1000 spores with a pathogenic field strain isolated from elephants in Zambia but died when challenged with a dose of 2500 spores. A technique was developed to identify anthrax organisms excreted with the faeces by means of gas chromatography.
Reuveny S, White MD, Adar YY, Kafri Y, Altboum Z, Gozes Y, Kobiler D, Shafferman A, Velan B. (2001). Search for correlates of protective immunity conferred by anthrax vaccine. Infect Immun. 69(5): 2888-2893	Department of Biotechnology, Israel Institute for Biological Research, Ness-Ziona 74100, Israel. Vaccination by anthrax protective antigen (PA)-based vaccines requires multiple immunization, underlying the need to develop more efficacious vaccines or alternative vaccination regimens. In spite of the vast use of PA-based vaccines, the definition of a marker for protective immunity is still lacking. Here we describe studies designed to help define such markers. To this end we have immunized guinea pigs by different methods and monitored the immune response and the corresponding extent of protection against a lethal challenge with anthrax spores. Active immunization was performed by a single injection using one of two methods: (i) vaccination with decreasing amounts of PA and (ii) vaccination with constant amounts of PA that had been thermally inactivated for increasing periods. In both studies a

	direct correlation between survival and neutralizing-antibody titer was found (r(2) = 0.92 and 0.95, respectively). Most significantly, in the two protocols a similar neutralizing-antibody titer range provided 50% protection. Furthermore, in a complementary study involving passive transfer of PA hyperimmune sera to naive animals, a similar correlation between neutralizing-antibody titers and protection was found. In all three immunization studies, neutralization titers of at least 300 were sufficient to confer protection against a dose of 40 50% lethal doses (LD(50)) of virulent anthrax spores of the Vollum strain. Such consistency in the correlation of protective immunity with anti-PA antibody titers was not observed for antibody titers determined by an enzyme-linked immunosorbent assay. Taken together, these results clearly demonstrate that neutralizing antibodies to PA constitute a major component of the protective immunity against anthrax and suggest that this parameter could be used as a surrogate marker for protection.
Rhie GE, Roehrl MH, Mourez M, Collier RJ, Mekalanos JJ, Wang JY. (2003). A dually active anthrax vaccine that confers protection against both bacilli and toxins. Proc Natl Acad Sci U S A. 100(19): 10925-10930.	Department of Microbiology and Molecular Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. Systemic anthrax is caused by unimpeded bacillar replication and toxin secretion. We developed a dually active anthrax vaccine (DAAV) that confers simultaneous protection against both bacilli and toxins. DAAV was constructed by conjugating capsular poly-gamma-d-glutamic acid (PGA) to protective antigen (PA), converting the weakly immunogenic PGA to a potent immunogen, and synergistically enhancing the humoral response to PA. PGA-specific antibodies bound to encapsulated bacilli and promoted the killing of bacilli by complement. PA-specific antibodies neutralized toxin activity and protected immunized mice against lethal challenge with anthrax toxin. Thus, DAAV combines both antibacterial and antitoxic components in a single vaccine against anthrax. DAAV introduces a vaccine design that may be widely applicable against infectious diseases and provides additional tools in medicine and biodefense.
Rice EW, Adcock NJ, Sivaganesan M, Rose LJ (2005). Inactivation of Spores of *Bacillus anthracis* Sterne, Bacillus cereus, and Bacillus thuringiensis subsp. israelensis by Chlorination. Applied and Environmental Microbiology 71: 5587-5589	National Homeland Security Research Center, U.S. EPA, 26 W. M. L. King Dr., Cincinnati, OH 45268. Phone: (513) 569-7204. Fax: (513) 487-2555. E-mail: rice.gene@epa.gov Three species of Bacillus were evaluated as potential surrogates for *Bacillus anthracis* for determining the sporicidal activity of chlorination as commonly used in drinking water treatment. Spores of Bacillus thuringiensis subsp. israelensis were found to be an appropriate surrogate for spores of B. anthracis for use in chlorine inactivation studies.
Ristroph JD, Ivins BE. (1983). Elaboration of *Bacillus anthracis* antigens in a new, defined culture medium. Infection and Immunity.. 39: 1, 483-486.	Improved culture conditions and a new, completely synthetic medium (R medium) were developed for the production of B. anthracis holotoxin antigens. Levels of these antigens up to fivefold greater than the highest previously reported values were recovered with this system. Cultures of Sterne, V770-NP1-R, and Vollum 1B strains of B. anthracis were monitored for growth, pH change, glucose utilization, supernatant protein concentration, lethal toxin activity, and protease activity.
Robertson DL, Tippetts MT, Leppla SH. (1988). Nucleotide sequence of the Bacillus anthracis edema factor gene (cya): a calmodulin-dependent adenylate cyclase. Gene 73(2): 363-371	Department of Chemistry, Brigham Young University, Provo, UT 84602. The nucleotide sequence of the Bacillus anthracis edema factor (EF) gene (cya), which encodes a calmodulin-dependent adenylate cyclase, has been determined. EF is part of the tripartite protein exotoxin of B. anthracis. An ATG start codon, immediately upstream from codons which specify the first 15 amino acids (aa) of EF, was preceded by an AAAGGAGGT sequence which is its probable ribosome-binding site. Starting at this ATG codon, there was a continuous 2400-bp open reading frame which encodes the 800-aa EF-precursor protein with a Mr of 92,464. The mature, secreted protein (767 aa; Mr 88,808) was preceded by a 33-aa signal peptide which has characteristics in common with leader peptides for other secreted proteins of the Bacillus species. A consensus amino acid sequence (Gly-X-X-X-X-Gly-Lys-Ser,X = any aa), which was part of the presumed ATP binding site for EF, was also present. The codon usage of the EF gene reflected the high A + T (71%) base composition for its DNA. B. anthracis EF was not

	related to the Escherichia coli or yeast adenylate cyclases, but was related to the Bordetella pertussis calmodulin-dependent adenylate cyclase.
Robertson I, Owen J. (1994). Notifiable diseases of pigs. In Practice.. 16: 3, 110---126.	This article describes the clinical and, where appropriate, post mortem signs which might give rise to suspicion that a notifiable disease is present, taking into account the differential diagnosis.
Robertson I, Owen J. (1995). Notifiable disease of pigs. [Spanish] Veterinary Record (Editorial Espanol).. 8: 1, 30-46.	
Robson SL (2004). Anthrax. Agfact A0.9.24, third edition, August 2004, NSW Department of Primary Industries	'all carcases must be burned to minimise the spread of contamination'
Roffey R, Tegnell A, Elgh F. (2002). Biological warfare in a historical perspective. Clin Microbiol Infect 8: 450-454	
Romanov GI, Rudenko LP. (1980). Improving the quality control of "STI" anthrax vaccine by tests on laboratory animals. Trudy Vsesoyuznogo Gosudarstvennogo Nauchno-Kontrol'nogo Instituta Veterinarnykh Preparatov.. 29/30: 195-200.	
Romanov GI. (1980). Preparation, control and application of anthrax vaccine in USSR. Arch Exp Veterinarmed. 34(1):119-22.	Reported in this paper are the preparation, testing and application of two live vaccines against anthrax in the USSR. They were STI live vaccine in liquid and lyophilised forms and GHKI live vaccine in lyophilised form. Official tests for purity were conducted, using growth factors of cultures, whereas harmlessness was tested on rabbit and effectiveness on guinea-pig and later on sheep, after some five years had elapsed. Quantitative methods by which to test the effectiveness of VGNKI are now being prepared. Immunisation for prophylaxis or emergency are undertaken, depending on epizootiological situations. Major importance is ascribed to keeping, feeding, general nutrition and performance, latent infections, and meteorological factors which can all be of impact on immunogenicity and postvaccinal complications. Anthrax has been eliminated in the USSR, with only few sporadic outbreaks left, owing to immunisation and complementary action in veterinary hygiene.
Romic V, Balent M, Udovicic I. (1981). Experience in the production of a vaccine against blackleg, braxy and anthrax (3-VAK). Praxis Veterinaria.. 29: 3/4, 277-280.	[Serbo-Croatian]
Rose AL. (1940a). Pulmonary anthrax in a bullock. Aust Vet J 16: 213-214	"Seven days before this animal manifested symptoms [lesions confined to the thoracic cavity], the whole mob was stampeding round and worrying the carcase of a heifer, which had died, undoubtedly of anthrax, about 2 weeks previously."
Rose AL. (1940b). The period of incubation of anthrax in cattle. Aust Vet J 16: 214-	Incubation period of anthrax in cattle was observed to be 4-6 days

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Rose LJ, Rice EW, Jensen B, Murga R, Peterson A, Donlan RM, Arduino MJ. (2005). Chlorine inactivation of bacterial bioterrorism agents. Appl Environ Microbiol. 71(1): 566-568	Centers for Disease Control and Prevention, 1600 Clifton Rd., C16, Atlanta, GA 30333, USA. lrose@cdc.gov Seven species of bacterial select agents were tested for susceptibility to free available chlorine (FAC). Under test conditions, the FAC routinely maintained in potable water would be sufficient to reduce six species by 2 orders of magnitude within 10 min. Water contaminated with spores of *Bacillus anthracis* spores would require further treatment.
Rosovitz MJ, Schuck P, Varughese M, et al. (2003). Alanine-scanning mutations in domain 4 of anthrax toxin protective antigen reveal residues important for binding to the cellular receptor and to a neutralizing monoclonal antibody. J Biol Chem 278: 30936–44.	[Scobie et al 2005, 12]
Ross JM (1957). The pathogenesis of anthrax following the administration of spores by the respiratory route. J Pathol Bacteriol 73: 485–494	[Cote et al 2005]
Rowe-Taitt CA, Hazzard JW, Hoffman KE, Cras JJ, Golden JP, Ligler FS. (2000). Simultaneous detection of six biohazardous agents using a planar waveguide array biosensor. Biosensors & Bioelectronics.. 15: 11/12, 579-589.	It was recently demonstrated that an array biosensor could be used with cocktails of fluorescent antibodies to perform three assays simultaneously on a single substrate, and that multiple samples could be analysed in parallel. This technology was extended to demonstrate the simultaneous analysis of six samples for six different hazardous analytes, including both bacteria and protein toxins. The level of antibody cross-reactivity is explored, revealing a possible common epitope in two of the toxins. A panel of environmental interferents was added to the samples; these interferents neither prevented the detection of the analytes nor caused false-positive responses. Antibodies and antigens used were: cholera toxin (CT) (from Vibrio cholerae type Inaba); Staphylococcus aureus enterotoxin B (SEB); rabbit anti-cholera toxin immunoglobulin IgG; Ricin; *Bacillus anthracis* Sterne strain; Francisella tularensis LVS strain; killed Brucella abortus; and antibodies directed against B. anthracis, B. abortus, F. tularensis, ricin, and SEB.
Rubinstein E, Levi I. (2002). Biological warfare: implications for antimicrobial use. Current Infectious Disease Reports. BioMed Central. 4: 1, 28-34.	Biological warfare is intended to incapacitate a large number of individuals at a single exposure, creating epidemic-type disease, death, and social chaos. The organisms with potential for immediate use as bacteriologic weapons are *Bacillus anthracis*, Brucella melitensis, Yersinia pestis, and Vibrio cholera, all necessitating antibiotic therapy for a cure. It is reasonable, therefore, to assume that a biological attack, or even a hoax, would require thousands of individuals over a large area to begin antibiotic therapy. Issues such as antibiotic availability, logistical problems in antibiotic distribution, development of drug resistance, side effects influencing the individual, and adverse effects on the community due to the impact of mass therapy on the ecology, make biological warfare the most apocalyptic scenario for the creation of a "postantibiotic era".
Ryu ChunSun; Lee Kyunghee; Yoo Cheonkwon; Seong WonKeun; Oh HeeBok. (2003). Sensitive and rapid quantitative detection of anthrax spores isolated from soil samples by real-time	Quantitative analysis of anthrax spores from environmental samples is essential for accurate detection and risk assessment since *Bacillus anthracis* spores have been shown to be one of the most effective biological weapons. Using TaqMan real-time PCR, specific primers and probes were designed for the identification of pathogenic B. anthracis strains from pag gene and cap gene on two plasmids, pXO1 and pXO2, as well as a sap gene encoded on the S-layer. To select the appropriate lysis method of anthrax spore from environmental samples, several heat treatments and

PCR. Microbiology and Immunology.. 47: 10, 693-699.	germination methods were evaluated with multiplex-PCR. Among them, heat treatment of samples suspended with sucrose plus non-ionic detergent was considered an effective spore disruption method because it detected up to 105 spores/g soil by multiplex-PCR. Serial dilutions of B. anthracis DNA and spore were detected up to a level of 0.1 ng/ micro l and 10 spores/ml, respectively, at the correlation coefficient of 0.99 by real-time PCR. Quantitative analysis of anthrax spore could be obtained from the comparison between CT value and serial dilutions of soil sample at the correlation coefficient of 0.99. Additionally, spores added to soil samples were detected up to 104 spores/g soil within 3 h by real-time PCR. As a consequence, we established a rapid and accurate detection system for environmental anthrax spores using real-time PCR, avoiding time and labour-consuming preparation steps such as enrichment culturing and DNA preparation.
Sacchi CT, Whitney AM, Mayer LW, Morey R, Steigerwalt A, Boras A, Weyant RS, Popovic T. (2002). Sequencing of 16S rRNA gene: a rapid tool for identification of Bacillus anthracis. Emerg Infect Dis 8(10): 1117-1123	Centers for Desease Control and Prevention , Atlanta, Georgia 30333, USA. cls9@cdc.gov In a bioterrorism event, a tool is needed to rapidly differentiate Bacillus anthracis from other closely related spore-forming Bacillus species. During the recent outbreak of bioterrorism-associated anthrax, we sequenced the 16S rRNA generom these species to evaluate the potential of 16S rRNA gene sequencing as a diagnostic tool. We found eight distinct 16S types among all 107 16S rRNA gene seqs fuences that differed from each other at 1 to 8 positions (0.06% to 0.5%). All 86 B. anthracis had an identical 16S gene sequence, designated type 6; 16S type 10 was seen in all B. thuringiensis strains; six other 16S types were found among the 10 B. cereus strains. This report describes the first demonstration of an exclusive association of a distinct 16S sequence with B. anthracis. Consequently, we were able to rapidly identify suspected isolates and to detect the B. anthracis 16S rRNA gene directly from culture-negative clinical specimens from seven patients with laboratory-confirmed anthrax.
Sadovoi NV, Derbin MI, Garin NS, Tarumov VS, Mikhailov VV, Shentsev IV, Fofanov PY. (1979). Preparation and properties of anthrax protective antigen. V. Dynamics of immunity after immunization with concentrated, purified, chemical adsorbed anthrax vaccine. Zhurnal Mikrobiologii Epidemiologii i Immunobiologii.. 5, 64-67.	
Saggar SN, Joseph MM, Bell WJ. (1974). Treatment of cutaneous anthrax (in man) with a single oral dose of doxycycline. East African Medical Journal.. 51: 12, 889-894.	
Sala V, Piccininno G, Ciuchini F, Pistoia C. (1982). First production trials of an anthrax anti-toxin vaccine for veterinary use. Atti della Societa Italiana delle Scienze Veterinarie.. 36: 569-571.	[Italian]
Salles II, Tucker AE, Voth DE, Ballard JD. (2003). Toxin-induced resistance in *Bacillus anthracis* lethal toxin-treated	

macrophages PNAS 100: 12426-12431	
Salmon DD, Ferrier GR. (1991). Post vaccination occurrence of anthrax in cattle. Veterinary Record.. 130: 7, 140-141.	Post vaccination deaths caused by anthrax on 2 properties in southern New South Wales, Australia are reported. Both properties were in the 'anthrax belt' but anthrax had not been diagnosed previously on either property. On the first property of 120 lactating dairy cattle, all the cattle were vaccinated with an anthrax spore vaccine following the death of a cow from anthrax. Three more cows died between 11 and 126 days after vaccination; anthrax was confirmed in 2 cases. The herd was revaccinated and no more cattle died. On the second property, the herd of 200 beef cattle was vaccinated with spore vaccine after 3 of 43 cattle in one paddock died and anthrax had been confirmed. Two deaths occurred 3 and 5 days after vaccination. The cattle in the same paddock were treated with antibiotics, moved to another paddock and revaccinated; 37 days later a heifer died who had not been treated with antibiotic and anthrax was confirmed. The entire herd was revaccinated and no more cattle died. Possible reasons for the post vaccination deaths are discussed. ELISA showed low Ab level before vaccination and 4 fold increase after vaccination. Local strain of B anthracis may have unusual antigenic qualities and require different vaccination approach. Use of penicillin-streptomycin.
Saltykov RA, Bakulov IA, Gavrilov VA, Ulanova AA, Kivaev VA. (1976). Characteristics of the anthrax vaccinal strain STI-1 preserved for 30 years in the form of lyophilized spores. Zh Mikrobiol Epidemiol Immunobiol. (6):62-5.	A study was made of the biological properties of the spore culture of anthrax vaccine strain STI-1 lyophilized in 1944 and kept for 30 years without any passages. This dry culture contained not less than 29% of live spores; the culture growth in the nutrient broth and on the agar medium was typical for the strain. Immunogenicity tested in experiments on guinea pigs and rabbits was not reduced and corresponded to that of the reference STI-1 vaccine.
Samad MA, Hoque ME. (1986). Anthrax in man and cattle in Bangladesh. J Trop Med Hyg;89:43-45.	
Samer Singh; Aparna Singh; Mohd. Azhar Aziz; Syed Mohsin Waheed; Rajiv Bhat; Rakesh Bhatnagar. (2004). Thermal inactivation of protective antigen of *Bacillus anthracis* and its prevention by polyol osmolytes. Biochemical and Biophysical Research Communications.. 322: 3, 1029-1037.	Protective antigen (PA) of *Bacillus anthracis* is the main immunogen of all anthrax vaccines. It is a highly thermolabile molecule and loses its activity rapidly when exposed to higher temperatures. Earlier some cosolvents had been used to stabilize PA with variable success but no study has been done to find out the primary cause of PA thermal inactivation. This study aims at elucidating the predominant cause of thermal inactivation of PA in order to develop more effective strategies for its thermostabilization. The prime cause for the loss of biological activity of PA at high temperature was its aggregation and an inverse correlation between PA activity and its aggregation on heating was observed. Inactivation of the protein by autolysis did not occur. This paper reports the use of a series of polyol osmolytes to stabilize PA. Different polyols stabilized PA to a different extent against thermal inactivation in a concentration dependent manner, with glycerol stabilizing to the maximum extent. Addition of NaCl to glycerol solution further enhanced the thermal stability of PA. An increase in the T1/2 value, the temperature at which 50% of the activity is retained during short-term incubation, of more than 20 degrees C was observed. The half-life (t1/2) of PA thermal inactivation at 40 degrees C increased by more than 6 times in the presence of the mixture of glycerol and NaCl as compared to control. This study demonstrates for the first time that aggregation of the PA molecule is the predominant cause of its thermal inactivation, and can be very effectively prevented by the use of glycerol and other polyols to increase the shelf life of the recombinant vaccine against anthrax.
Samer Singh; Aziz, M. A, Puneet	Protective antigen (PA) is the main immunogenic constituent of all vaccines against anthrax. It is known to lose its

Khandelwal; Rajiv Bhat; Rakesh Bhatnagar. (2004). The osmoprotectants glycine and its methyl derivatives prevent the thermal inactivation of protective antigen of *Bacillus anthracis*. Biochemical and Biophysical Research Communications.. 316: 2, 559-564.	biological activity even at 37 degrees C. Its thermolabile nature has, thus, remained a cause of concern as even transient exposure of the vaccine to higher temperature could compromise its efficacy. Various types of cosolvent excipients have been used to stabilize a number of proteins with variable success. However, no comprehensive and systematic study to stabilize anthrax PA molecule using this approach has ever been undertaken. We have carried out a systematic study on the effect of osmoprotectants like glycine and its methyl derivatives, sarcosine, dimethylglycine, and betaine, on the thermostability of PA. The thermal stability of PA was found to be highly sensitive to pH with maxima at pH 7.9. All the cosolvent additives used were able to enhance the thermal stability of PA as inferred from an increase in T1/2 values, the temperature at which 50% activity was retained during short-term incubation. Glycine was found to be the best stabilizer, while the ability of its methyl derivatives to stabilize PA decreased with an increase in the number of substituted methyl groups suggesting perturbation of hydrophobic interactions. On extended incubation at 40 degrees C the half-life of PA thermal inactivation increased more than four times in the presence of glycine. Thus, glycine could be used as an effective stabilizer to enhance the shelf life of recombinant vaccine against anthrax.
Sanderson WT, Hein MJ, Taylor L, Curwin BD, Kinnes GM, Seitz TA, Popovic T, Holmes HT, Kellum ME, McAllister SK, Whaley DN, Tupin EA, Walker T, Freed JA, Small DS, Klusaritz B, Bridges JH (2002). Surface Sampling Methods for *Bacillus anthracis* Spore Contamination. Emerg Infect Dis 8: 1145-1151	During an investigation conducted December 17–20, 2001, we collected environmental samples from a U.S. postal facility in Washington, D.C., known to be extensively contaminated with *Bacillus anthracis* spores. Because methods for collecting and analyzing B. anthracis spores have not yet been validated, our objective was to compare the relative effectiveness of sampling methods used for collecting spores from contaminated surfaces. Comparison of wipe, wet and dry swab, and HEPA vacuum sock samples on nonporous surfaces indicated good agreement between results with HEPA vacuum and wipe samples. However, results from HEPA vacuum sock and wipe samples agreed poorly with the swab samples. Dry swabs failed to detect spores >75% of the time when they were detected by wipe and HEPA vacuum samples. Wipe samples collected after HEPA vacuum samples and HEPA vacuum samples collected after wipe samples indicated that neither method completely removed spores from the sampled surfaces.
Santelli E, Bankston LA, Leppla SH, Liddington RC. (2004). Crystal structure of a complex between anthrax toxin and its host receptor. Nature 430: 905-908	
Sasaki J, Kita J. (2003). Bacteriocidal activity of garlic powder against *Bacillus anthracis*. J Nutr Sci Vitaminol (Tokyo). 49(4):297-9.	Department of Medical Technology, Hirosaki University School of Health Sciences, Honcho, Hirosaki 036-8564, Japan. jisasaki@cc.hirosaki-u.ac.jp The antibacterial activity of garlic powder was examined against *Bacillus anthracis* using agar plate cultivation and test tube methods. On the agar plate test, 1-5% garlic powder inhibited the growth of B. anthracis and Escherichia coli O157 used as references. A 1% water solution of garlic powder in the test tube method killed B. anthracis at 10(7) cfu/mL within 3 h of treatment at room temperature. A number of intestinal bacteria in a BALB/c mouse decreased after the oral administration of 1 mL of 1%, garlic powder solution once a day for 3 d. These results suggest that the oral administration of garlic powder is effective against pathogenic bacteria invasion into the intestine as an infection.
Sawada-Hirai R, Jiang I, Wang F, Sun SM, Nedellec R, Ruther P, Alvarez A, Millis D, Morrow PR, Kang AS. (2004). Human anti-anthrax protective antigen neutralizing monoclonal antibodies derived from donors vaccinated with anthrax vaccine adsorbed. J Immune	Avanir Pharmaceuticals Inc, Antibody Technology, 11388 Sorrento Valley Rd, San Diego, California 92121, USA. akang@avanir.com BACKGROUND: Potent anthrax toxin neutralizing human monoclonal antibodies were generated from peripheral blood lymphocytes obtained from Anthrax Vaccine Adsorbed (AVA) immune donors. The anti-anthrax toxin human monoclonal antibodies were evaluated for neutralization of anthrax lethal toxin in vivo in the Fisher 344 rat bolus toxin challenge model. METHODS: Human peripheral blood lymphocytes from AVA immunized donors were engrafted into severe combined immunodeficient (SCID) mice. Vaccination with anthrax protective antigen and lethal factor produced

Based Ther Vaccines. May 12;2(1):5.	a significant increase in antigen specific human IgG in the mouse serum. The antibody producing lymphocytes were immortalized by hybridoma formation. The genes encoding the protective antibodies were rescued and stable cell lines expressing full-length human immunoglobulin were established. The antibodies were characterized by; (1) surface plasmon resonance; (2) inhibition of toxin in an in vitro mouse macrophage cell line protection assay and (3) in vivo in a Fischer 344 bolus lethal toxin challenge model. RESULTS: The range of antibodies generated were diverse with evidence of extensive hyper mutation, and all were of very high affinity for PA83~1 x 10-10-11M. Moreover all the antibodies were potent inhibitors of anthrax lethal toxin in vitro. A single IV dose of AVP-21D9 or AVP-22G12 was found to confer full protection with as little as 0.5x (AVP-21D9) and 1x (AVP-22G12) molar equivalence relative to the anthrax toxin in the rat challenge prophylaxis model. CONCLUSION: Here we describe a powerful technology to capture the recall antibody response to AVA vaccination and provide detailed molecular characterization of the protective human monoclonal antibodies. AVP-21D9, AVP-22G12 and AVP-1C6 protect rats from anthrax lethal toxin at low dose. Aglycosylated versions of the most potent antibodies are also protective in vivo, suggesting that lethal toxin neutralization is not Fc effector mediated. The protective effect of AVP-21D9 persists for at least one week in rats. These potent fully human anti-PA toxin-neutralizing antibodies are attractive candidates for prophylaxis and/or treatment against Anthrax Class A bioterrorism toxins.
Schlingman AS, Devlin HB, Wright GG, Maine RJ, Manning MC (1956) Immunizing activity of alum-precipitated protective antigen of *Bacillus anthracis* in cattle, sheep and swine. Am J Vet Res 17: 256-261	Protective antigen Sheep were solidly immune three months after challenge. Cattle were solidly immune one month after immunization and immunity declined but ws still significant after 8 and 12 months. Challenge by oral route superior to systemic route.
Schmid G, Kaufmann A (2002). Anthrax in Europe: its epidemiology, clinical characteristics, and role in bioterrorism. Clin Microbiol Infect 8: 479-488	
Schneerson R, Kubler-Kielb J, Liu TY, Dai ZD, Leppla SH, Yergey A, Backlund P, Shiloach J, Majadly F, Robbins JB. (2003). Poly(gamma-D-glutamic acid) protein conjugates induce IgG antibodies in mice to the capsule of *Bacillus anthracis*: a potential addition to the anthrax vaccine. Proc Natl Acad Sci U S A. 100(15):8945-50.	National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA. schneerr@mail.nih.gov Both the protective antigen (PA) and the poly(gamma-d-glutamic acid) capsule (gamma dPGA) are essential for the virulence of *Bacillus anthracis*. A critical level of vaccine-induced IgG anti-PA confers immunity to anthrax, but there is no information about the protective action of IgG anti-gamma dPGA. Because the number of spores presented by bioterrorists might be greater than encountered in nature, we sought to induce capsular antibodies to expand the immunity conferred by available anthrax vaccines. The nonimmunogenic gamma dPGA or corresponding synthetic peptides were bound to BSA, recombinant B. anthracis PA (rPA), or recombinant Pseudomonas aeruginosa exotoxin A (rEPA). To identify the optimal construct, conjugates of B. anthracis gamma dPGA, Bacillus pumilus gamma dLPGA, and peptides of varying lengths (5-, 10-, or 20-mers), of the d or l configuration with active groups at the N or C termini, were bound at 5-32 mol per protein. The conjugates were characterized by physico-chemical and immunological assays, including GLC-MS and matrix-assisted laser desorption ionization time-of-flight spectrometry, and immunogenicity in 5- to 6-week-old mice. IgG anti-gamma dPGA and antiprotein were measured by ELISA. The highest levels of IgG anti-gamma dPGA were elicited by decamers of gamma dPGA at 10 -20 mol per protein bound to the N-

	or C-terminal end. High IgG anti-gamma dPGA levels were elicited by two injections of 2.5 microg of gamma dPGA per mouse, whereas three injections were needed to achieve high levels of protein antibodies. rPA was the most effective carrier. Anti-gamma dPGA induced opsonophagocytic killing of B. anthracis tox-, cap+. gamma dPGA conjugates may enhance the protection conferred by PA alone. gamma dPGA-rPA conjugates induced both anti-PA and anti-gamma dPGA.
Schuch R, Nelson D, Fischetti VA (2002). A bacteriolytic agent that detects and kills *Bacillus anthracis*. Nature 418: 884-889	[Cherwonogrodzky 2005, 37]
Schwartz MN (2001). Recognition and management of anthrax – an update. N Engl J Med 345: 1621-1626	[Brachman et al 2004, 23]
Scobie HM, Rainey GJ, Bradley KA, Young JA. (2003). Human capillary morphogenesis protein 2 functions as an anthrax toxin receptor. Proc Natl Acad Sci USA 100: 5170–4.	[Scobie et al 2005, 4]
Scobie HM, Thomas D, Marlett JM, Destito G, Wigelsworth DJ, Collier RJ, Young JAT, Manchester M (2005). A Soluble Receptor Decoy Protects Rats against Anthrax Lethal Toxin Challenge. Journal of Infectious Diseases 192: 1047–1051	Successful postexposure treatment for inhalation anthrax is thought to include neutralization of anthrax toxin. The soluble anthrax toxin receptor/tumor endothelial marker 8 and capillary morphogenesis protein 2 (sATR/TEM8 and sCMG2, respectively) receptor decoys bind to anthrax toxin protective antigen (PA) and compete with cellular receptors for binding. Here, we show that, in a tissue-culture model of intoxication, sCMG2 is a 11.4-fold more potent antitoxin than sATR/TEM8 and that this increased activity corresponds to an ~1000-fold higher PA-binding affinity. Stoichiometric concentrations of sCMG2 protect rats against lethal toxin challenge, making sCMG2 one of the most effective anthrax antitoxins described to date.
Scobie HM, Young JAT. (2005). Interactions between anthrax toxin receptors and protective antigen. Current Opinion in Microbiology.. 8: 1, 106-112.	Since the anthrax mail attacks of 2001, much has been learned about the interactions between anthrax toxin and its receptors. Two distinct cellular receptors for anthrax toxin have been identified and are designated capillary morphogenesis protein 2 (CMG2) and anthrax toxin receptor/tumor endothelial marker 8 (ATR/TEM8). The molecular details of the toxin-receptor interactions have been revealed through crystallographic, biochemical and genetic studies. In addition, a novel pathway by which anthrax toxin enters cells is starting to be uncovered.
Seddon HR (1953). Anthrax. *In* Diseases of domestic animals in Australia. Part 5. Volume 1. Commonwealth of Australia, Canberra, pp 8-40	
Seddon HR. (1965). Diseases of Domestic Animals in Australia. Part 5, Volume 1: Bacterial Diseases (2nd edition), Australian Department of Health, Commonwealth of Australia, Department of Health, Canberra, pp 12-26	
Sellman BR, Mourez M, Collier RJ. (2001).	The protective antigen moiety of anthrax toxin translocates the toxin's enzymic moieties to the cytosol of mammalian

Dominant-negative mutants of a toxin subunit: an approach to therapy of anthrax. Science . 292: 5517, 695-697.	cells by the mechanism that depends on its ability to heptamerize and insert into membranes. We identified dominant-negative mutants of protective antigen that co-assemble with the wild-type protein and block its ability to translocate the enzymic moieties across membranes. These mutants strongly inhibited toxin action in cell culture and a animal intoxication model, suggesting that they could be useful in therapy of anthrax.
Selyaninov Yu O, Balyshev VM, Kosyachenko NS, Egorova I Yu. (2002). Experimental assessment of the efficacy of associated vaccine against anthrax and sheep pox. Russian Agricultural Sciences., publ. 2003. 7, 43-45.	This study was conducted to evaluate the intensity of immunity induced by anthrax and pox components of an associated vaccine by experimental infection of sheep with pathogenic strains, including cross infection. Nine Romanov sheep were used in this study. After vaccination of the experimental sheep, no changes were observed at the site of injection. The general clinical state and appetite of sheep were normal. The anti-anthrax antibody titres in the blood serum of the experimental sheep varied from 1:80-1:320, whereas that of anti-pox varied from 1:4-1:8. The blood sera of intact animals did not contain anti-anthrax and virus neutralizing antibodies. Two control animals infected with anthrax became ill and died on the 2nd and 3rd days, respectively. Control sheep no. 8 infected with the virulent Mongolian pox virus strain became ill on the 5th day and showed characteristic clinical signs. After cross infection with virulent strains in both groups, no increase in body temperature was observed and clinical manifestations of anthrax and sheep pox were absent during the entire observation period. The associated vaccine against anthrax and sheep pox developed is an immunologically valuable preparation. The immunological response of the organism to each component of the associated preparation does not differ from that obtained with separate vaccination using monopreparations. Moreover, the lyophilized associated preparation retains its immunobiological properties for at least a year if stored at 4-6 degrees C.
Sen SK, Minett FC. (1944). Experiments on the transmission of anthrax. Indian Journal of Veterinary Science and Animal Husbandry;14:149-158.	
Sergeant E (1999). Anthrax. Animal Health Surveillance (Newsletter of Australia's National Animal Health Information System) 4(4): 9	Positive smears for anthrax in case in sheep in Bourke RLPB where 100/1700 sheep were found dead, in an area where anthrax had occurred previously.
Seto K. (1981). Vaccines for veterinary use (XV). 16. Anthrax vaccine. Journal of the Japan Veterinary Medical Association.. 34: 4, 172-174.	
Sever JL, Brenner AI, Gale AD, Lyle JM, Moulton LH, Ward BJ, West DJ. (2004). Safety of anthrax vaccine: an expanded review and evaluation of adverse events reported to the Vaccine Adverse Event Reporting System (VAERS). Pharmacoepidemiol Drug Saf. 13(12):825-40.	Department of Pediatrics, Obstetrics and Gynecology, The Children's National Medical Center, George Washington University, Washington, DC 20010-2970, USA. jsever@cnmc.org PURPOSE: To assess the safety of a licensed anthrax vaccine (AVA) given to more than 500,000 US military personnel, through review and medical evaluation of adverse events (AEs) reported to the Vaccine Adverse Event Reporting System (VAERS). METHODS: AEs were summarized by person, vaccine lot, type, frequency and impact. A Delphic approach was used to tentatively assess causality in an effort to detect serious AEs (SAEs) or other medically important AEs (OMIAEs) possibly attributable to AVA. RESULTS: The Anthrax Vaccine Expert Committee (AVEC) reviewed 1841 reports describing 3991 AEs (9.4 reports/10,000 doses of AVA) that were submitted to VAERS from 1Q1998 through 4Q2001. One hundred forty-seven reports described an SAE or OMIAE, of which 26 were tentatively

	rated as possible, probable or certain consequences of vaccination (injection-site reaction [12], 'anaphylactic-like reaction' [5] and eight other systemic AEs [1-2 each]). CONCLUSIONS: This review produced no evidence for an unusual rate of any SAE or OMIAE attributable to AVA. It supported an earlier impression that AVA may cause significant local inflammation and should be administered over the deltoid rather than the triceps to avoid direct or compression injury to the ulnar nerve. The subjects of VAERS reports tended to be older than all recipients of AVA. Females generally had and/or reported AEs more often than males, but transient articular reactions were surprisingly more common in males. Variations in the frequency or severity (as judged by hospitalization and/or loss of duty) of reported AEs did not suggest a significant problem with (1) a particular lot of AVA, (2) recurrent AEs after multiple doses or (3) vaccination of persons with a concomitant illness or those given other vaccines or medications. Copyright 2004 John Wiley & Sons, Ltd.
Sever JL, Brenner AI, Gale AD, Lyle JM, Moulton LH, West DJ; Anthrax Vaccine Export Committee. (2002). Safety of anthrax vaccine: a review by the Anthrax Vaccine Expert Committee (AVEC) of adverse events reported to the Vaccine Adverse Event Reporting System (VAERS). Pharmacoepidemiol Drug Saf. 11(3):189-202.	Departments of Pediatrics, Obstetrics and Gynecology, Microbiology and Immunology, Children's National Medical Center, George Washington University, School of Medicine, 111 Michigan Avenue, N.W., Washington, DC 20010-2970, USA. jsever@cnmc.org PURPOSE: To assess the safety of a licensed anthrax vaccine given to nearly 400,000 US military personnel, reports of adverse events (AEs) submitted to the Vaccine Adverse Event Reporting System (VAERS) were reviewed and evaluated medically. METHODS: The Anthrax Vaccine Expert Committee (AVEC), a civilian panel of private-sector physicians and other scientists, reviewed 602 VAERS reports using a Delphic approach (structured expert consensus) to assess the causal relationship between vaccination and the reported AEs and sought to identify unexpected patterns in the occurrence of medically important events. Reports were entered into a database and used to profile AEs with respect to person, type/location, relative frequency, severity/impact, concomitant illness or receipt of other drugs or vaccines, and vaccine lot. RESULTS: Nearly half the reports noted a local injection-site AE, with more than one-third of these involving a moderate to large degree of inflammation. Six events qualified as serious AEs (SAEs), and all were judged to be certain consequences of vaccination. Three-quarters of the reports cited a systemic AE (most common: flu-like symptoms, malaise, rash, arthralgia, headache), but only six individual medically important events were judged possibly or probably due to vaccine (aggravation of spondyloarthropathy (2), anaphylactoid reaction, arthritis (2), bronchiolitis obliterans organizing pneumonia). CONCLUSIONS: Since some cases of local inflammation involved distal paresthesia, AVEC recommends giving subcutaneous injections of AVA over the inferior deltoid instead of the triceps to avoid compression injury to the ulnar nerve. At this time, ongoing evaluation of VAERS reports does not suggest a high frequency or unusual pattern of serious or other medically important AEs.
Shangkuan YH, Yang JF, Lin HC, Shaio MF. (2000). Comparison of PCR-RFLP, ribotyping and ERIC-PCR for typing *Bacillus anthracis* and Bacillus cereus strains. Journal of Applied Microbiology.. 89: 3, 452-462.	PCR-RFLP analysis of the vrrA gene and cerAB gene was used to investigate the genomic diversity in 21 strains of *Bacillus anthracis* and 28 strains of Bacillus cereus, and compared with results obtained by ribotyping and enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) analysis. VrrA-typing divided the B. anthracis into four groups. Except for one Pasteur vaccine strain, the vrrA PCR-RFLP profiles of the B. anthracis were separated into three groups, which were different from those of the B. cereus strains. Ribotyping separated the B. anthracis isolates into seven ribotypes, and a common fragment of an approximately 850 bp band from the ERIC-PCR fingerprints separated most B. anthracis strains into two groups. VrrA/cerAB PCR-RFLP, ribotyping and ERIC-PCR generated 18, 22 and 23 types, respectively, from B. cereus strains. The results suggest that a combination of all three methods provides a high resolution typing method for B. anthracis and B. cereus. Compared with ribotyping and ERIC-PCR, PCR-RFLP is simple to perform and has potential as a rapid method for typing and discriminating B. anthracis strains from other B. cereus group bacteria.
Shentsev IV, Shumilov GP, Sadovoi NV,	

Derbin MI, Mikhailov VV. (1981). Storage life of live anthrax vaccines prepared with liquid and solid culture media. Zhurnal Mikrobiologii Epidemiologii i Immunobiologii.. 7, 113-114.	
Shentsev IV, Shumilov GP, Tarumov VS, Derbin MI. (1978). An index of blood neutrophil injury in man and animals immunized with live anthrax vaccine. Zh Mikrobiol Epidemiol Immunobiol. (6):81-4.	The authors present the results of study of the blood neutrophil injury in guinea pigs, rabbits, monkeys, and also humans inoculated subcutaneously with live anthrax vaccine. Along with intradermal test with anthraxin, the mentioned test is suggested to assess the immunological status of persons immunized with anthrax vaccine.
Shepard CW, Soriano-Gabarro M, Zell ER, Hayslett J, Lukacs S, Goldstein S, Factor S, Jones J, Ridzon R, Williams I, Rosenstein N; CDC Adverse Events Working Group. (2002). Antimicrobial postexposure prophylaxis for anthrax: adverse events and adherence. Emerg Infect Dis. 8(10):1124-32.	Centers for Desease Control and Prevention , Atlanta, Georgia 30333, USA. cvc8@cdc.gov We collected data during postexposure antimicrobial prophylaxis campaigns and from a prophylaxis program evaluation 60 days after start of antimicrobial prophylaxis involving persons from six U.S. sites where *Bacillus anthracis* exposures occurred. Adverse events associated with antimicrobial prophylaxis to prevent anthrax were commonly reported, but hospitalizations and serious adverse events as defined by Food and Drug Administration criteria were rare. Overall adherence during 60 days of antimicrobial prophylaxis was poor (44%), ranging from 21% of persons exposed in the Morgan postal facility in New York City to 64% of persons exposed at the Brentwood postal facility in Washington, D.C. Adherence was highest among participants in an investigational new drug protocol to receive additional antibiotics with or without anthrax vaccine--a likely surrogate for anthrax risk perception. Adherence of <60 days was not consistently associated with adverse events.
Shewsbury JFD, Barson GJ. (1952). A bacteriological study of house sparrows, Passer domesticus, domesticus. J Pathol Bacteriol 64: 605	
Shiferaw F, Abditcho S, Gopilo A, Laurenson MK. (2002). Anthrax outbreak in Mago National Park, southern Ethiopia. Veterinary Record.. 150: 10, 318-320.	This paper describes two outbreaks of anthrax in wildlife in Mago National Park. On 29 September 1999 sudden deaths were first reported in lesser kudu (Tragelaphus strepsiceros) in the south of the park. The epidemic spread north during the next 2 months and 1615 fatal cases were recorded in 21 species. Lesser kudu were the most severely affected ungulate with 95% of recorded deaths and approximately 67% of the population dying. Deaths also included primates, carnivores and birds. Postmortem examinations of kudu revealed an oral frothy discharge and blood oozing from the nose, mouth and anus which did not clot. Blood, spleen and lung smear sample analysis yielded *Bacillus anthracis*. Previously, deaths in domestic cattle, sheep and goats were reported in the nearby Karo village in 27 September 1999 but was limited by vaccination. However, livestock entering Mago Park continued to die. 20 people suffered skin lesions after handling or eating affected carcasses but also recovered after treatment. During September and October 2000, another outbreak occurred in the park. Although lesser kudu were again the most severely affected species, there were substantially fewer deaths than in 1999. While the origins of these outbreaks are unclear, livestock encroachment into the park should be controlled and avoided for conservation reasons.
Shiferaw G. (2004). Anthrax in Wabessa village in the Dessie Zuria district of Ethiopia. Revue Scientifique et Technique - Office International des	In 2002 an investigation of sudden death in a goat in Wabessa village in the Dessie Zuria district of Ethiopia was undertaken using fresh blood brought to the Kombolcha Regional Veterinary Laboratory. The sample was examined using standard bacteriological techniques and animal pathogenicity tests were also performed. The laboratory investigation revealed *Bacillus anthracis* as the cause of sudden death. Information gathered from stockowners in the

Epizooties. 23: 3, 951-956.	same village revealed other similar recent cases and deaths, both in animals and humans, with farmers clearly describing the clinical signs and necropsy findings of anthrax. The disease occurs annually in this area in May and June, and in the 2002 outbreak mortality rates of 7.7%, 32.7% and 47.1% were observed in cattle, goats and donkeys, respectively. This study indicates that the community of this particular village neither knows of, nor practises, any of the conventional methods for anthrax control. The cutaneous form of the disease in humans and the environmental contamination associated with the practise of opening cadavers are briefly described and the findings are discussed with reference to the epidemiology of anthrax in both Ethiopia and elsewhere. Control strategies are also recommended.
Shi-Hua Wang, Ji-Kai Wen, Ya-Feng Zhoua, Zhi-Ping Zhang Rui-Fu Yang, Ji-Bin Zhang Jia Chena, Xian-En Zhang. (2004) Identification and characterization of *Bacillus anthracis* by multiplex PCR on DNA chip. Biosensors & Bioelectronics 20 807–813	*Bacillus anthracis* can be identified by detecting virulence factor genes located on two plasmids, pXO1 and pXO2. Combining multiplex PCR with arrayed anchored primer PCR and biotin–avidin alkaline phosphatase indicator system, we developed a qualitative DNA chip method for characterization of B. anthracis, and simultaneous confirmation of the species identity independent of plasmid contents. The assay amplifies pag gene (in pXO1), cap gene (in pXO2) and Ba813 gene (a B. anthracis specific chromosomal marker), and the results were indicated by an easy-to-read profile based on the color reaction of alkaline phosphatase. About 1 pg of specific DNA fragments on the chip wells could be detected after PCR. With the proposed method, the avirulent (pXO1+/2−, pXO1−/2+ and pXO1−/2−) strains of B. anthracis and distinguished 'anthrax-like' strains from other B. cereus group bacteria were unambiguously identified, while the genera other than Bacillus gave no positive signal.
Shinn AH, Bravo NC, Maecker HT, Smith JW. (2003). TNF-alpha detection using a flow cytometric assay to determine cellular responses to anthrax vaccine. J Immunol Methods. 282(1-2): 169-174	Thomas J Long School of Pharmacy, University of the Pacific, Stockton, CA 95211, USA. hshinn@uop.edu This study describes a four-color flow cytometric assay that detects CD4+ T cell responses to the anthrax vaccine. Whole blood from seven volunteers who previously obtained the anthrax vaccine was inoculated in vitro with varying concentrations of the anthrax antigen. TNF-alpha and IFN-gamma production from memory CD4+ T cells were measured and compared to a control group who never received the anthrax vaccine. The optimal antigen concentration for TNF-alpha was determined to be around 7.5 microg/ml and IFN-gamma production was not detected. This assay will be used in future larger prospective studies to further evaluate the cellular immune responses induced by the anthrax vaccine.
Shlyakhov E, Blancou J, Rubinstein E (1996). Vaccines against anthrax in animals, from Louis Pasteur to our day. Revue scientifique et technique Off Int Epizoot 15: 853-862	The authors outline the history of vaccination against anthrax in animals, from the end of the 19th century to the present time. The three main steps in the production of specific vaccines are described in detail: production of vaccines from live, encapsulated bacteria, followed by vaccines from live, unencapsulated bacteria and, finally, subunit vaccines. Advantages and disadvantages of these three types of vaccine, some of which are still in use today, are described and discussed.
Shlyakhov E, Blancou J, Rubinstein E. (1996). Les vaccins contre la fievre charbonneuse des animaux, de Louis Pasteur a nos jours. Rev Sci Tech OIE 15: 853-862	
Shlyakhov E, Blancou J, Segev S, Rubinstein E. (1998). 100 years of research on immune response to *Bacillus anthracis*. Annales de Medecine Veterinaire. 142: 2, 101-110.	

Shlyakhov E, Gruz EV, Prisaker' VI. (1980). Optimization of anthrax vaccination on the basis of rational classification of populated sites. Zh Mikrobiol Epidemiol Immunobiol. (4):91-5.	The improved epidemiological situation requires the proper revision of the tactics used in vaccinating the population against anthrax. The important task lies in the optimization of the immunoprophylaxis of the rural population in accordance with the epizootologic situation in a given locality. To ensure correct orientation in choosing the groups of population to be vaccinated a rational classification of inhabited localities is proposed: they are classified as anthrax-free and anthrax-affected, and the latter fall into manifest, conditionally manifest, nonmanifest and unclassified. The use of the cartographic method based on this classification is recommended. The proposed vaccination tactics will allow to reduce the number of persons covered by immunoprophylaxis by 70-75%, making this coverage more exact, and to improve the epidemiological effectiveness of vaccination.
Shlyakhov E, Rubinstein E, Novikov I. (1997). Anthrax post-vaccinal cell-mediated immunity in humans: kinetics pattern. Vaccine. 15(6-7): 631-636	Infectious Diseases Unit, Sheba Medical Center, Sackler School of Medicine, Tel-Aviv University, Tel Hashomer, Israel. Seven groups (2596 subjects) were vaccinated with a human live anthrax vaccine (HLAV) by three different routes (scarification, subcutaneous and aerosol). The vaccinees were tested for anthrax cell-mediated immunity using the "Anthraxin" skin test at 7, 15, 30, 90, 180 and 365 days following vaccination. The kinetic pattern obtained from all groups, shows a significant, five-phased curve: phase I (2-6 days post-vaccination) shows a slow increase in positive Anthraxin skin reactions. Phase II (7-15 days post-vaccination) shows an exponential rise to a maximum at day 15. Phase III (16-30 days post-vaccination) shows a decrease to day 30. Phase IV (31-90 days post-vaccination) leads to a relative restoration of the positive skin reactions. During phase V (91-365 days post-vaccination) there is a continuous decrease in positive Anthraxin skin reactions. The loss of the skin test reaction on day 30 is a characteristic feature of post vaccination anthrax cell-mediated immunity. It may be due to a blockade of macrophages by lethal anthrax toxin released by the multiplying vaccine strain. Epidemiological observations of HLAV protective rates correlate with the phases of the skin reaction kinetics.
Shlyakhov E, Rubinstein E. (1994). Delayed hypersensitivity after anthrax vaccination. I. Study in guineapigs vaccinated against anthrax. Medecine Tropicale.. 54: 1, 33-37.	An anthraxin allergy test was performed in 682 guineapigs at various times after immunization with veterinary unencapsulated active anthrax vaccine. Results were compared with those obtained in 216 unimmunized control guineapigs, in 183 guineapigs that received a non-immunizing dose of virulent vaccine and in 120 guineapigs inoculated with inactive vaccine. Anthraxin allergy tests were positive in the 1st days after vaccination. The incidence and intensity of positive tests peaked between 2 weeks and 1 month after vaccination and then gradually decreased during the 1st year. Study of resistance of guineapigs to an inoculum at a lethal dose of a virulent strain of *Bacillus anthracis* showed a close correlation between positive tests and resistance. It is suggested that anthraxin allergy test could have practical applications for the production of vaccines and for evaluation of the immune status of vaccinated pets.
Shlyakhov E, Rubinstein E. (1994). Human live anthrax vaccine in the former USSR. Vaccine. 12(8):727-30.	Infectious Diseases Unit, Sheba Medical Centre, Sackler School of Medicine, Tel-Aviv University, Tel-Hashomer, Israel. The history of the development and use of the Soviet live spore human anthrax vaccine is described. Results of mass field trials on this vaccine following administration by scarification, by subcutaneous injection route or by aerosol exposure are presented. For the immunological assessment of these vaccinations a skin test with an original product 'Anthraxin' was used.
Shlyakhov E, Rubinstein E. (1995). Rapid bioassay for detection of *Bacillus anthracis* in mice. Journal of Veterinary Medicine. Series B.. 42: 6, 361-368.	A rapid-bioassay method (RBA) of diagnosing anthrax was developed in mice. A standard RBA consisted of intraperitoneal inoculation of a suspected culture or of a suspension of raw infected animal tissues in 4 pairs of mice. At 60, 120 and 180 min after inoculation, 2 mice were killed and smears and impression smears were made from the peritoneal fluid, heart blood, spleen, liver and kidneys, and then fixed, stained and microscopically examined. Encapsulated rods seen in microscopy confirmed the diagnosis. The remaining pair of mice was kept for a 10-day observation period and served as a control. In the experiments described, RBA showed a high efficiency. A vegetative culture of 18-20 h of B. anthracis growth demonstrated encapsulation in mice as early as 120 min after inoculation; a culture of 3-5 or 6-12 h of growth showed encapsulation at 60 min. Old spores gave encapsulation at 180 min, but, if

	previously plated for 6 h, encapsulation was observed as early as 60 min after inoculation. Inoculation of raw infected tissues gave positive results at 120 min. As compared with the challenged controls, no deviations between the results of RBA and the final diagnosis of anthrax were observed, but RBA appears to be 7-17 times more rapid. Some variations of the standard RBA are also suggested.
Shlyakhov E, Rubinstein E. (1996). Anthraxin skin testing: an alternative method for anthrax vaccine and post-vaccinal immunity assessment. Journal of Veterinary Medicine. Series B.. 43: 8, 483-488.	The use of the anthraxin skin test in guineapigs is described and recommended as a non-aggressive, immunological test for the potency of live veterinary anthrax vaccines.
Shlyakhov E, Shoenfeld Y, Gilburd B, Rubinstein E. (2004). Evaluation of *Bacillus anthracis* extractable antigen for testing anthrax immunity. Clinical Microbiology and Infection. 10: 5, 421-424.	Three extractable *Bacillus anthracis* cell wall-associated antigens were evaluated for potential use as skin testing agents, and as possible candidates for in vitro diagnosis of anthrax immunity. Anthraxin and a partially purified extractable antigen (EAP) were produced from avirulent B. anthracis strain 34F2 (Sterne). The thermoextractable antigen used for the Ascoli reaction was obtained commercially. Guineapigs were immunized and boosted several times subcutaneously with the Sterne live veterinary anthrax vaccine. Four weeks after the last booster dose, animals were skin-tested with the three antigens. Serum antibody levels were also determined by ELISA, and the in vitro T cell response was evaluated by [3H]-thymidine incorporation. EAP was the most active antigen in both the serological and cellular reactions. EAP also elicited a distinct positive skin reaction in animals immunized with B. anthracis. The data obtained in this preliminary study indicated that extractable cell wall antigens obtained from the vegetative form of B. anthracis may be used for skin tests and in vitro testing of specific humoral and cell-mediated anthrax immunity.
Shlyakov E. (1996). Anthraxin: a skin test for early and retrospective diagnosis of anthrax and anthrax vaccination assessment. Salisbury Medical Bulletin, special supplement #87, June, p. 109	
Shoop WL, Xiong Y, Wiltsie J, Woods A, Guo J, Pivnichny JV, Felcetto T, Michael BF, Bansal A, Cummings RT, Cunningham BR, Friedlander AM, Douglas CM, Patel SB, Wisniewski D, Scapin G, Salowe SP, Zaller DM, Chapman KT, Scolnick EM, Schmatz DM, Bartizal K, MacCoss M, Hermes JD. (2005). Anthrax lethal factor inhibition. Proceedings of the National Academy of Sciences of the United States of America. . 102: 22, 7958-7963.	The primary virulence factor of *Bacillus anthracis* is a secreted zinc-dependent metalloprotease toxin known as lethal factor (LF) that is lethal to the host through disruption of signaling pathways, cell destruction, and circulatory shock. Inhibition of this proteolytic-based LF toxemia could be expected to provide therapeutic value in combination with an antibiotic during and immediately after an active anthrax infection. Herein is shown the crystal structure of an intimate complex between a hydroxamate, (2R)-2-[(4-fluoro-3-methylphenyl)sulfonylamino]-N-hydroxy-2-(tetrahy dro-2H-pyran-4-yl)acetamide, and LF at the LF-active site. Most importantly, this molecular interaction between the hydroxamate and the LF active site resulted in (i) inhibited LF protease activity in an enzyme assay and protected macrophages against recombinant LF and protective antigen in a cell-based assay, (ii) 100% protection in a lethal mouse toxemia model against recombinant LF and protective antigen, (iii) ~50% survival advantage to mice given a lethal challenge of B. anthracis Sterne vegetative cells and to rabbits given a lethal challenge of B. anthracis Ames spores and doubled the mean time to death in those that died in both species, and (iv) 100% protection against B. anthracis spore challenge when used in combination therapy with ciprofloxacin in a rabbit "point of no return" model for which ciprofloxacin alone provided 50% protection. These results indicate that a small molecule, hydroxamate LF inhibitor, as revealed herein, can ameliorate the toxemia characteristic of an active B. anthracis infection and could be a vital adjunct to our ability to combat anthrax.

Sidel FR, Takafuji ET, Franz DF. (Eds.) (1997). Medical Aspects of Chemical and Biological Warfare. Office of the Surgeon General, Department of the Army. Accessible on The Virtual Naval Hospital: http://www.vnh.org/MedAspChemBioWar	
Sidel V, Cohen HW, Gould RM (2002). From woolsorter's to mail sorters: anthrax past, present and future. Am J Public Health 92: 705-706	
Singh Y, Chaudhary VK, Leppla SH. (1989). A deleted variant of *Bacillus anthracis* protective antigen is non-toxic and blocks anthrax toxin action in vivo. J Biol Chem. 264(32):19103-7.	Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-5011. Anthrax toxin is the only protein secreted by *Bacillus anthracis* that contributes to the virulence of this bacterium. An obligatory step in the action of anthrax toxin on eukaryotic cells is cleavage of the receptor-bound protective antigen (PA) protein (83 kilodaltons) to produce a 63-kilodalton, receptor-bound COOH-terminal fragment. A similar fragment can be obtained by limited treatment with trypsin. This proteolytic processing event exposes a site with high affinity for the other two anthrax toxin proteins, lethal factor and edema factor. Terminal sequencing of the purified fragment showed that the activating cleavage occurred in the sequence Arg164-Lys165-Lys166-Arg167. The gene encoding PA was mutagenized to delete residues 163-168, and the deleted PA was purified from a Bacillus subtilis host. The deleted PA was not cleaved by either trypsin or the cell-surface protease, and was non-toxic when administered with lethal factor. Purified, deleted PA protected rats when administered 90 min before injection of 20 minimum lethal doses of toxin. This mutant PA may be useful as a replacement for the PA that is the major active ingredient in the current human anthrax vaccine, because deleted PA is expected to have normal immunogenicity, but would not combine with trace amounts of LF and EF to cause toxicity.
Singh Y, Ivins BE, Leppla SH. (1998). Study of immunization against anthrax with the purified recombinant protective antigen of *Bacillus anthracis*. Infect Immun. 66(7):3447-8.	Centre for Biochemical Technology, Delhi, India. Protective antigen (PA) of anthrax toxin is the major component of human anthrax vaccine. Currently available human vaccines in the United States and Europe consist of alum-precipitated supernatant material from cultures of toxigenic, nonencapsulated strains of *Bacillus anthracis*. Immunization with these vaccines requires several boosters and occasionally causes local pain and edema. We previously described the biological activity of a nontoxic mutant of PA expressed in Bacillus subtilis. In the present study, we evaluated the efficacy of the purified mutant PA protein alone or in combination with the lethal factor and edema factor components of anthrax toxin to protect against anthrax. Both mutant and native PA preparations elicited high anti-PA titers in Hartley guinea pigs. Mutant PA alone and in combination with lethal factor and edema factor completely protected the guinea pigs from B. anthracis spore challenge. The results suggest that the mutant PA protein may be used to develop an effective recombinant vaccine against anthrax.
Sirisanthana T, Brown AE (2002). Anthrax of the gastrointestinal tract. Emerg Infect Dis 8: 649-651	
Sirisanthana T, Nelson KE, Ezzell J, Abshire TG (1988). Serological study of patients	[Brachman et al 2004, 124]

with cutaneous and oral-oropharyngeal anthrax from northern Thailand. Am J Trop Med Hyg 39: 575-581	
Siromakova M, Valev V, Cvetanov J, Mireva J (1975). Comparative immunological and morphological studies with the protective antigen of Bacillus anthracis. Journal of Biological Standardization 3(1): 31-32	Medical Academy, Sofia, Bulgaria. Purification of PA and examination of immunological and immunochemical properties. Morphological changes in regional lymph nodes and parenchymal organs of experimental animals elucidated with purified PA and compared with responses to live STI-1 vaccine.
Slobodyan VV. (1997). Influence of Antrakol vaccine against anthrax on the immune system. Visnik Agrarnoi Nauki.. 5, 79-80.	[Ukrainian] The immune response of calves vaccinated against anthrax with strain K-79Z vaccine (Antrakol) was examined and compared with that of non-vaccinated controls (in total 82 calves). Haematological analyses revealed that leukocyte count and lymphocyte percentage were significantly higher in the vaccinated calves (12.3+or-0.35 x 109/litre and 69.30+or-0.70%, respectively) compared with the controls (9.0+or-0.23 x 109/litre and 61.33+or-0.66%). Neutrophil phagocytic activity was 50% higher in the vaccinated calves compared with the controls. An increase of 11% in lysozyme activity was observed in vaccinated calves. Endogenous interferon was determined in blood serum 4 years after vaccination. It is concluded that Antrakol vaccine stimulates both cellular and humoral immunity.
Smith H (1990). Anthrax, clinical manifestations and pathogenesis: much unknown and much just forgotten. Proceedings of the international workshop on anthrax. Salisbury Medical Bulletin;68(suppl):31.	
Smith H (2000). Discovery of the anthrax toxin: the beginning of in vivo studies on pathogenic bacteria. Trends Microbiol 8: 199-200	
Smith H, Keppie J (1954). Observations on experimental anthrax: demonstration of a specific lethal factor produced in vivo by Bacillus antracis. Nature 173: 860-870	First description of a toxin responsible for death, discerned from studies in guinea pigs where specific anthrax antiserum neutralized its effects.
Smith H, Keppie J, Stanley JL, Harris-Smith PW. (1955). The chemical basis of the virulence of *Bacillus anthracis*. IV: Secondary shock as the major factor in death from anthrax. Br J Exp Pathol 136:323-335.	
Smith H, Keppie J, Stanley JL, Harris-Smith PW. (1955). The chemical basis of the virulence of *Bacillus anthracis*. V: The	A toxin produced by B anthracis is responsible for secondary shock and death. The toxin produces oedema in the skin and kills mice and guinea pigs. Both these effects are neutralised by anthrax antiserum.

specific toxin produced by B. anthracis in vivo. Br J Exp Pathol 136: 460-472	The toxin is unrelated to the capsule. Close relationship between the toxin and the non-toxic protective antigen.
Smith KA (2005). Wanted, an anthrax vaccine: dead or alive? Medical Immunology 4: 5	
Smith KL, de Vos V, Bryden HB, Hugh-Jones ME, Klevytska A, Price LB, Keim P, Scholl DT. (1999). Meso-scale ecology of anthrax in southern Africa: a pilot study of diversity and clustering. J Appl Microbiol 87: 204-207	
Sotoodehnia A, Aarabi I, Ale-agha S, Mahinpour M, Naserirad A, Hamedi M. (1992). Study on a combined anthrax-tetanus vaccine for immunization of equines. Archives de l'Institut Razi.. 42/43, 69-73.	
Sotoodehnia A, Aarabi I. ((1988). Neutralization of excess formalin by sodium meta-bisulfite in combined anthrax and clostridial vaccine. Archives de l'Institut Razi.. 38/39, 89-91.	
Sotoodehnia A, Aarabi I. (1984). The comparison of two anthrax spore vaccines prepared with Sterne 34F2 and native C5 strains in sheep and goats in Iran. Archives de l'Institut Razi.. 34/35: 51-54.	As a result of tests on sheep, goats and guinea pigs, the avirulent, Iranian strain C5 of *Bacillus anthracis* was replaced by the uncapsulated, attenuated Sterne strain 34F2 for vaccine production at the Razi Institute, Iran.
Spears HN, Davidson JC. (1959). Anthrax. Vet Rec 71: 637-643	Review of incidence, aetiology, susceptibility, diagnosis, treatment, prevention and control measures in Great Britain. 1938-1958: 9,452 outbreaks. Cattle followed by pigs mainly affected, sheep a distant third Feedingstuffs main source of infection (3,584/4,668 outbreaks in period 1952-1958) Seldom that more than one animal dies in each outbreak. Penicillin and chlortetracycline used with considerable success. Need for an effective sterile antigen vaccine. Dairy cattle vaccinated with Sterne vaccine have drop in milk yield. Some farmers vaccinate and then resume use of anthrax contaminated feed.
Spencer RC. (2003). *Bacillus anthracis*. J Clin Pathol. 56(3): 182-187	Public Health Laboratory, Bristol Royal Infirmary, UK. robert.spencer@ubht.swest.nhs.uk The events of 11 September 2001 and the subsequent anthrax outbreaks have shown that the West needs to be prepared for an increasing number of terrorist attacks, which may include the use of biological warfare. *Bacillus anthracis* has long been considered a potential biological warfare agent, and this review will discuss the history of its

	use as such. It will also cover the biology of this organism and the clinical features of the three disease forms that it can produce: cutaneous, gastrointestinal, and inhalation anthrax. In addition, treatment and vaccination strategies will be reviewed.
Spencer TL, McKenzie FM, Ferrier GR. (1994). ELISA to study antibody responses to anthrax vaccine in cattle, sheep and goats. Strengthening research on animal reproduction and disease diagnosis in Asia through the application of immunoassay techniques: Proceedings of the Final Research Co-ordination Meeting, Bangkok, Thailand, 1-5 February 1993.. International Atomic Energy Agency (IAEA), Vienna, Austria:. 177-184.	DPI Victoria, RVL Benalla Cattle (1ml), sheep (0.5ml) and goats (0.5ml) vaccinated with CSL vaccine (10million spores/ml) at 0 and 6 (c &s) or 0 and 22 weeks (s & g). Response to vaccination monitored by regular ELISA tests. Dramatic variation in responses between animals. Anamnestic responses observed. Suggested that variability due to individual animal immune responses and that it should be tested by challenge studies (with appropriate containment).
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Stanley E. (1892). Anthrax in animals. Agric Gazette NSW 3: 527-528	Outreak of anthrax in the Parramatta district – a farmer, not suspecting the nature of the disease, had a valuable cow that had died skinned and boiled for the pigs.
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1950. Vet Med 46: 141-142	
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Stein CD (1954). Anthrax in livestock during 1953 and a review of data on incidence from 1945 to 1953. Vet Med 49: 277	
Stein CD (1963). Anthrax. In: Hull TG, ed. Diseases transmitted from animals to man. Springfield, IL: Charles C. Thomas,;82-121.	
Stein CD, Stoner MG (1952). Anthrax in livestock during 1951 and comparative data on the disease from 1945 through 1951. Vet Med 47: 315-320	
Stein CD, Van Ness GB. (1955). A ten year survey of anthrax in livestock with special reference to outbreaks in 1954. Vet Med 50: 579	
Stepanov AS, Marinin LI, Staritsyn NA et al: (1997). Molecular mechanisms underlying *Bacillus anthracis* infection at early stages and search for novel vaccines. Vestn Ross Akad Med Nauk 6:16,	
Stepanov AV, Marinin LI, Pomerantsev AP, Staritsin NA. (1996). Development of novel vaccines against anthrax in man. J Biotechnol. 44(1-3):155-60.	State Research Institute of Applied Microbiology, Obolensk, Russia. It has been shown that antianthrax immunity induced by the novel vaccine proposed has not only antitoxic, but also antispore character. The whole complex of antigens, namely surface spore antigens, surface antigens of cell wall and toxin components is required for the induction of strong and stable immunity against anthrax. The STI-1 vaccine strain with introduced resistance to several antibiotics seems to be promising for prophylaxis and treatment of anthrax in case of emergency, especially if antibiotic pretreatment could be expected. The technology for submerged cultivation of *Bacillus anthracis* vaccine strain and for the development of an anthrax vaccine to be used in human medicine is proposed on the basis of the conception of the immunogenesis.
Sternbach G. (2003). The history of anthrax. Journal of Emergency Medicine. . 24: 4, 463-467.	Anthrax, a potentially fatal infection, is a virulent and highly contagious disease. Descriptions of this disease begin in antiquity, with the best ancient account being by the Roman poet Virgil. During the 19th century, anthrax was the infection involved in several important medical developments. It served as the prototype for Koch's postulates regarding the causation of infectious disease. The first vaccine containing attenuated live organisms was Louis Pasteur's veterinary anthrax vaccine. In the 1900s, human inhalation anthrax occurred sporadically in the United States among textile and tanning workers, but the incidence of the illness had declined dramatically. An outbreak of inhalation anthrax

	occurred in Sverdlovsk near a Soviet military microbiology facility in 1979. This epidemic represented the largest documented outbreak of human inhalation anthrax in history. In October and November 2001, 22 cases of confirmed or suspected inhalation and cutaneous anthrax were reported associated with the intentional release of the organism in the United States. An additional case of cutaneous disease occurred in March of 2002.
Sterne M (1937a). Variation in *Bacillus anthracis*. Onderstepoort J Vet Sci Anim Ind 8: 271-349	
Sterne M (1937b). The effects of different carbon dioxide concentrations on the growth of virulent anthrax strains. Pathogenicity and immunity tests on guinea-pigs and sheep with anthrax variants derived from virulent strains. Onderstepoort J Vet Sci Anim Ind 9: 49-67	'even strains which produced a relatively poor immunity in guinea-pigs were comparatively successful when tried on sheep' 'differences in growth were noted with different batches of media and sera' 'the usual dose [300,000 spores] of this strain did not produce a strong immunity in guinea-pigs or sheep, but when then times this concentration [3 million spores] was given the sheep acquired a solid immunity' 'it appears probable that larger doses of potent avirulent strains may give maximal immunity to anthrax'
Sterne M (1939a) The use of anthrax vaccines prepared from avirulent (uncapsulated) variants of *Bacillus anthracis*. Onderstepoort J Vet Sci Anim Ind 13: 307-312	300,000 spores per cc. Strain $34F_2$ was isolated from a virulent strain on 10.8.36. Cattle dose: 1cc containing 600,000 to 1.2 million spores. From mid 1938, suspended in 0.5% saponin in 50% glycerine-saline
Sterne M (1939b) The immunization of laboratory animals against anthrax. Onderstepoort J Vet Sci Anim Ind 13: 313-317	Mice less susceptible than guinea pigs to a test dose of 100 guinea pig MLD of a Pasteur II strain.
Sterne M (1946). Avirulent anthrax vaccine. Onderstepoort J Vet Sci Anim Ind 21: 41-43	'the dose of vaccine used is one cubic centimetre, containing about 10 million spores.'
Sterne M (1948a). The effect of inflammation on the survival of guinea pigs infected with anthrax. Onderstepoort J Vet Sci Anim Ind 23: 157-164	
Sterne M (1948b). The effect of inflammation on the development of immunity to anthrax in guinea pigs. Onderstepoort J Vet Sci Anim Ind 23: 165-169	Advantages of 20% NaCl solution as an excipient.

Sterne M (1959) Anthrax. *In* Infectious Diseases of Animals, Diseases due to Bacteria, Vol 1, Edited by AW Stableforth and IA Galloway, Butterworths Scientific Publications, London, pages 16- 52	"In spite of the resistance of the anthrax spore, it is not correct to visualize an almost indefinite persistence of pasture contamination. In dry soil, where microbial activity is minimal, anthrax spores can live for very long periods. Under the more usual conditions of competition with other organisms, anthrax contamination may disappear in a few months and rarely persists for longer than 3 or 4 years.""
Sterne M (1961). Anthrax. *In* Veterinary Annual, 3rd year, edited by WA Pool, John Wright & Sons, Bristol, pp 70-73	
Sterne M (1966). Anthrax. *In* Dalling T, ed. International encyclopedia of veterinary medicine Edinburgh: W Green and Son, Ltd., pages 221-230.	
Sterne M (1967) Distribution and economic importance of anthrax. Federation Proceedings 26: 1493-1495	Vaccine can be produced in deep culture at 10^5 doses per litre. A single vaccination provides 1 years protection but no more. Notes that Gladstone's sterile anthrax filtrate without an adjuvant was as potent an immunogen in guinea pigs as a good living spore vaccine. [Br J Exp Path 29: 379, 1948] Concurrent treatment and vaccination not advised [Lee et al Vet Rec 73: 1426, 1961]
Sterne M (1988). Anthrax vaccines. J Am Vet Med Assoc 192: 141.	
Sterne M (1989) Anthrax vaccination. Vet Rec 125(5): 118	Letter commenting on ADRs in vaccinated llamas – some species such as goats are known to be sensitive and the vaccine should be tested in any non-labelled species. Vaccines without saponin may be safer.
Sterne M, Nicol J, Lambrechts MC (1942). The effect of large scale active immunization against anthrax. J South African Vet Med Assoc 13: 53-63.	1923 – 7,500 deaths diagnosed in SA due to anthrax (real incidence possibly 5-10x higher) 1925+ - around 6 million doses of vaccine used per annum 1928 – first trial of 'block' vaccination by Nicol (all cattle in district vaccinated) 1941 - <700 anthrax deaths in all of SA. In none of 17 reported outbreaks were there >1 death.
Sterne M, Nicol J, Lambrechts MC. (1945). Field results with avirulent anthrax vaccine. J South African Vet Med Assoc 16: 12-13	Steady improvement in anthrax position in South Africa. Comparison: 1943-44: England 275 confirmed cases*; SAfr 188 cases Since 1939 all vaccine in SAfr made from Sterne strain * Vet Rec 56(2): 1944
Sterne M, Proom H. (1957). Induction of motility and capsulation in *Bacillus anthracis*. J Bacteriol 74: 541-542	No unequivocal evidence of motility in isolates identified as B anthracis. Further, no transformation of B anthracis from a genotypically noncapsulated to a capsulated state has yet been effected.

Sterne M, Robinson EM (1939). The preparation of anthrax spore vaccines (for cattle and sheep) in South Africa. Onderstepoort J Vet Sci Anim Ind 12: 9-18	'More batches are actually started but get discarded at different stages of manufacture.' 'It must be emphasized that laboratory results give only a general indication of the way a vaccine will behave in the field, and there is no substitute for the large scale testing of new batches' Notes on post vaccination accidents
Sterne M, Robinson EM, Nicol J (1939). The use of saponin spore vaccine for inoculation against anthrax in South Africa. Onderstepoort J Vet Sci Anim Ind 12: 279-302	Review of adjuvants. 'it was shown … that immunity to anthrax is closely bound up with the number bacilli in the vaccinating dose.' 'Laboratory experiments on sheep showed conclusively that saponin improved immunity.' 'A large scale field test failed to show any differences between saponin vaccine and ordinary spore vaccine.'
Sterne M. (1945). The action of saponin and other excipients on the virulence and immunizing of anthrax strains. J South African Vet Med Assoc 16: 53-58	"Saponin, apart from its tendency to foam is excellent, glycerine is not sufficiently irritant, concentrated NaCl [20%] may well prove the best of the lot." Other anthrax excipients described in literature: alum and agar; lanolin and olive oil
Sterne M. (1982). Anthrax island. Nature 295: 362	
Stevenson WJ, Hughes KL (19). Synopsis fo zoonoses in Australia. 2nd edition, Australian Governement Publishing Service, Canberra.	
Stewart JD. (1943). The advent of vaccination against anthrax in Australia. Aust Vet J 19: 148-149	"Dr Rougier's laboratory, which was well equipped, ws located at Double Bay, Sydney, and in addition to the manufacture of anthrax vaccine he became interested in the preparation of smallpox vaccine."
Stewart-Tull DES, Feavers IM, Gibson GR. (1999). 3rd International Conference on Anthrax, University of Plymouth, 7-10 September 1998. Journal of Applied Microbiology.. 87: 2, 187-321.	This issue contains 37 full papers and abstracts of papers covering the global anthrax situation, epidemiology of anthrax in Australia, South Africa and Canada, immunology and immunization against anthrax, diagnosis and detection of *Bacillus anthracis* and its toxins, and molecular aspects of B. anthracis and anthrax.
Stone SE. (1868). Cases of malignant pustule. Boston Medical and Surgical Journal;I:19–21.	[Macher 2002] First report of occupational anthrax in US
Streatfield SJ (2005). Plant-based vaccines for animal health. Rev Sci Tech OIE 24: 189-199	
Sugg RS (1948). Penicillin in treatment of anthrax. JAVMA 113: 467	1million units used in 8 clinically affected pyrexic calves who all recovered
Sussman HE. (2003). Spinach makes a safer anthrax vaccine. Drug Discov Today. 8(10):428-30.	

Sutton GD (1947). Avirulent anthrax vaccine. J South Afr Vet Med Assoc 18: 79-81	Strain 34F2 loses its immunogenic capacity when stored onmedia at room temperature or in the refrigerator. Loss is complete after 30 days. Some brands of casein produce unsatisfactory vaccine production.
Swarbrick O. (1967). Three incidents of anthrax. Vet Rec 80: 84	
Swartz MN. (2001). Recognition and management of anthrax—An update. N. Engl. J. Med. 345 (22), 1621– 1626.	[post-exposure antibiotic prophylaxis]
Tan Y, Hackett NR, Boyer JL, Crystal RG. (2003). Protective immunity evoked against anthrax lethal toxin after a single intramuscular administration of an adenovirus-based vaccine encoding humanized protective antigen. Human Gene Therapy. 14: 17, 1673-1682.	Because of the need to develop a vaccine to rapidly protect the civilian population in response to a bioterrorism attack with *Bacillus anthracis*, we designed AdsechPA, a replication-deficient human serotype 5 adenovirus encoding B. anthracis protective antigen (PA) with codons optimized for expression in mammalian cells. With a single intramuscular administration to mice of 109 particle units of AdsechPA, a dose that can be scaled to human use, anti-PA antibodies were evoked more rapidly and at a higher level than with a single administration of the new U.S. military recombinant PA/Alhydrogel vaccine. Importantly, AdsechPA afforded approximately 2.7-fold more protection than the recombinant PA vaccine against B. anthracis lethal toxin challenge 4 weeks after a single vaccination. Even at 11 days postvaccination, AdsechPA provided some survival benefit, whereas the rPA/Alhydrogel vaccine provided none. In the context that equivalent human doses of Ad vectors have already been demonstrated to be safe in humans, a single administration of AdsechPA may provide the means to rapidly protect the civilian population against B. anthracis in response to a bioterrorism attack.
Tanner WB, Potter ME, Teclaw RF, Kaufmann AF, Bilderback WR, Dorris KD, Patton CM. (1979). Public health aspects of anthrax vaccination of dairy cattle. Journal of the American Veterinary Medical Association.. 173: 11, 1465-1466.	A study was conducted to determine whether cows shed *Bacillus anthracis* in their milk following vaccination with the Sterne strain of B. anthracis. The study group consisted of 49 vaccinated and 6 non-vaccinated cows in a single herd. Following vaccination, blood samples were collected daily for 7 days, and milk samples were collected twice daily for 10 days, B. anthracis was not isolated from any of the blood or milk samples. As measured by the indirect haemagglutination test (Ab against PA), the cows had a low-level but definite immunologic response to vaccination.
Teixeira M. (1981). Anthrax in Portugal. Bulletin de l'Office International des Epizooties.. 93: 9/10, 1309-1317.	[French] brief history is presented of anthrax in man and animals in Portugal from 1932 to 1975, with tables, maps and graphs, including its geographical distribution, vaccination campaigns, morbidity and mortality rates, and final control.
Teshale EH, Painter J, Burr GA, Mead P, Wright SV, Cseh LF, Zabrocki R, Collins R, Kelley KA, Hadler JL, Swerdlow DL, members of the Connecticut Anthrax Response Team (2002). Environmental Sampling for Spores of *Bacillus anthracis*. Emerg Infect Dis 8: 1083-1087	On November 11, 2001, following the bioterrorism-related anthrax attacks, the U.S. Postal Service collected samples at the Southern Connecticut Processing and Distribution Center; all samples were negative for *Bacillus anthracis*. After a patient in Connecticut died from inhalational anthrax on November 19, the center was sampled again on November 21 and 25 by using dry and wet swabs. All samples were again negative for B. anthracis. On November 28, guided by information from epidemiologic investigation, we sampled the site extensively with wet wipes and surface vacuum sock samples (using HEPA vacuum). Of 212 samples, 6 (3%) were positive, including one from a highly contaminated sorter. Subsequently B. anthracis was also detected in mail-sorting bins used for the patient's carrier route. These results suggest cross-contaminated mail as a possible source of anthrax for the inhalational anthrax patient in Connecticut. In future such investigations, extensive sampling guided by epidemiologic data is imperative.
Thomas L (2002). Anthrax. Royal Society of New Zealand Gamma Series, February 2002, pp 1-5	Brief review of anthrax generally and with reference to its absence in NZ.

Thompson KM, Armstrong RE, Thompson DF (2005). Bayes, bugs, and bioterrorists. Lessons learned from the anthrax attacks. Center for Technology and National Security Policy, National Defense University. Available at http://www.ndu.edu/inss/press/nduphp.html	The U.S. government continues to improve its plans for protecting civilians and soldiers from attacks with biological weapons. Part of this effort focuses on developing strategies that recognize the difficult choices to be made in using and deploying resources. This paper presents a risk- and decision-based framework—derived from the field of Bayesian statistics—for developing strategies that facilitate managing the risks of biological agents. The framework recognizes the significantly different attributes of potential biological weapons and offers a strategy for improving communication to effectively coordinate national biopreparedness efforts. The framework identifies generic decisions related to routine immunization, response planning, stockpiling vaccines and therapeutic agents, surveillance choices, containment, emergency response training, research, media and communications preparations, information management, and policy development. This paper provides a straw man to be used in wargames, exercises, practices, etc., at all levels of government. Given the attention on anthrax following the 2001 attacks, this paper applies the framework to managing the risks of anthrax to provide an illustrative example. The example demonstrates that by organizing information at this level, decisionmakers can quickly understand the critical connections between different options (e.g., vaccinating with a new vaccine requires an investment in research; research might increase the opportunities for breaches of containment). With respect to managing the risks of an attack with anthrax, this analysis suggests the need for creation of a comprehensive national management plan that includes quantitative evaluation of resource investments. The authors conclude that the government should adopt a process—based on decision science and using the power of decision trees as an analytical tool—to develop a strategy for managing the risks of bioterrorism. Using this type of approach, the government can better characterize the costs, risks and benefits of different policy options and ensure the integration of policy development. Additionally, confirmed use and refinement of decision trees during exercises will provide analysis of the long-term consequences of decisions made during an event and give policymakers insights to improve initial decisions.
Tigertt WD. (1980). Anthrax. William Smith Greenfield, M.D., F.R.C.P., Professor Superintendent, the Brown Animal Sanatory Institution (1878-81). Concerning the priority due to him for the production of the first vaccine against anthrax. J Hyg (Lond). 85(3):415-20.	The purpose of this paper is to draw attention to the fact that W. S. Greenfield, working at the Brown Animal Sanatory Institution in London, prepared an effective vaccine against anthrax and described his results some months before the experiment of Pasteur at Pouilly-le-fort. Partly through lack of financial support and partly due to opposition by the antivivisectionists, Greenfield was forced to confine his experiments to a small number of animals, but his results were nevertheless conclusive. He showed that by continuous subculture in a fluid medium that the anthrax bacillus progressively lost its virulence, until it was harmless even to the most susceptible animal, the mouse. The injection of suitably attenuated organisms into cattle rendered them immune to the subsequent injection of virulent anthrax bacilli. Greenfield's work has been overlooked or neglected, and he has never received the credit due him. It is only fitting that his work should be acknowledged in the centenary of the year in which it was described. The following account is composed primarily of quotations from his published papers. For additional information on Greenfield, reference may be made to the series of papers by Wilson (1979 a, b). It may be pointed out that the method of attenuating the virulence of bacilli recorded by Pasteur in relation to the bacillus of fowl cholera was, like that of anthrax vaccine, anticipated by Greenfield.
Titball RW, Turnbull PCB, Hutson RA. (1991). The monitoring and detection of *Bacillus anthracis* in the environment. Symposium Series - Society for Applied Bacteriology.. 20, 9S-18S.	'Although it is frequently stated in texts that spores of B anthracis may survive in the environment for long periods, there are surprisingly few well-documented studies.' 'In most soils it appears that following germination of the spores, emergent vegetative cells of B anthracis are rapidly out-competed by other members of the soil microflora and the bacterium is eliminated within a few years.' 'Replication of B anthracis in the field is yet to be demonstrated' Gruinard Island: contamination detected over less than 2% of the island.

	Formaldehyde and disinfection. Deterministic model of anthrax epizootic (Hahn 1983)
Todd JH. (1992). Adaptation to environment--the Pasteur anthrax vaccine in Australia. Aust Vet J. 69(12):318-321.	Pasteur vaccine: 4p/head for sheep (1894). Gunn vaccine: 2-2.5p/head for sheep (1894) dropping to 1.5p in 1902
Torok TJ, Tauxe RV, Wise RP, Livengood JR, Sokolow R, Mauvais S, Birkness KA, Skeels MR, Horan JM, Foster LR. (1997). A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. J. Am. Med. Assoc. 278 (5), 389– 395.	[In 1984 members of the Rajneesh cult intentionally contaminated salad bars in several restaurants in The Dalles OR with Salmonella typhimurium to prevent voters from an election the outcome of which was expected to impact land use]
Toshkov A, Valerianov Ts. (1978). Rapid diagnostic culture of pathogenic bacteria from the environment. I. *Bacillus anthracis*. II. Agents of plague, glanders, melioidosis, brucellosis and tularaemia. Acta Microbiologica Bulgarica.. 1, 49-62.	To detect pathogenic bacteria in samples of water, soil and in washings from various objects, the authors applied the work of Schimmelbusch (1894, 1895), who observed hyperacute anthrax in mice after application of spores to a fresh wound of the tail. It did not work in the cases of glanders and brucellosis.
Traeger MS, Wiersma ST, Rosenstein NE, Malecki JM, Shepard CW, Raghunathan PL, Pillai SP, Popovic T, Quinn CP, Meyer RF, Zaki SR, Kumar S, Bruce SM, Sejvar JJ, Dull PM, Tierney BC, Jones JD, Perkins BA. (2002). First case of bioterrorism-related inhalational anthrax in the United States, Palm Beach County, Florida, 2001. Emerg. Infect. Dis. 8 (10), 1029–1034.	[On October 4 2001, the first case of bioterrorism-related anthrax was confirmed in a person in Palm Beach County FL.]
Trotta T, Losito S, Altamura SA, Chiocco D, Fasanella A. (2002). Anthrax. Vaccines for veterinary use. Obiettivi e Documenti Veterinari.. 23: 12, 7-11.	[Italian]
Tsydypov VTs, Gabdulvalieva GK. (1975). Intradermal tests with anthraxin in cattle vaccinated against or infected with *Bacillus anthracis*. Uchenye Zapiski Kazanskogo Veterinarnogo Instituta.. 119: 218-220.	
Tsydypov VTs, Speranskii VV, Olennikova II. (1989). Immune response of cows to	A year after inoculation of anthrax vaccine, 69 of 202 cattle possessed no anthrax antibody, while in 55 the HA titre was 1:30, and in 28 it was 1:60. A booster dose after one year induced antibodies in 51 of 60 cattle.

"STI" anthrax vaccine after previous vaccination. Veterinariya, Moscow.. No.2: 32.	
Tsydypov VTs, Speranskii VV. (1990). Correct dose of "STI" anthrax vaccine for revaccinating cattle. Veterinariya (Moskva).. 11, 23-24.	
Tsydypov VTs. (1976). Reactions to the ring-precipitation inhibition test in cows vaccinated with STI anthrax vaccine. Uchenye Zapiski Kazanskogo Veterinarnogo Instituta.. 122: 116-117.	The Russian STI anthrax vaccine was administered to 184 cows. The ring-precipitation inhibition test was used to monitor changes in antibody levels. The proportion of animals showing positive reactions to the test declined from 84% at 60 days after immunization to 60% at 270 days.
Tuchili LM, Pandey GS, Sinyangwe PF, Kaji T (1993). Anthrax in cattle, wildlife and humans in Zambia. Vet Rec 132: 487	Milk samples from outbreak on dairy farm in Lusaka were negative.
Turell MJ, Knudson GB (1987). Mechanical transmission of *Bacillus anthracis* by stable flies (Stomoxys calcitrans) and mosquitoes (Aedes aegypti and Aedes taeniorhynchus). Infect Immunol 55: 1859-61.	We evaluated the potential of stable flies, Stomoxys calcitrans, and two species of mosquitoes, Aedes aegypti and Aedes taeniorhynchus, to transmit *Bacillus anthracis* Vollum 1B mechanically. After probing on Hartley guinea pigs with a bacteremia of ca. 10(8.6) CFU of B. anthracis per ml of blood, individual or pools of two to four stable flies or mosquitoes were allowed to continue feeding on either uninfected guinea pigs or A/J mice. All three insect species transmitted lethal anthrax infections to both guinea pigs and mice. Both stable flies and mosquitoes transmitted anthrax, even when they were held at room temperature for 4 h after exposure to the bacteremic guinea pig before being allowed to continue feeding on the susceptible animals. This study confirms that blood-feeding insects can mechanically transmit anthrax and supports recent anecdotal reports of fly-bite-associated cutaneous human anthrax. The potential for flies to mechanically transmit anthrax suggests that fly control should be considered as part of a program for control of epizootic anthrax.
Turnbull PCB (1986). Thoroughly modern anthrax. Abstracts on Hygiene and Tropical Diseases.. 61: 9, R1-R13.	
Turnbull PCB (1990). Anthrax. In Topley & Wilson's principles of bacteriology, virology and immunity, 8th edition, Volume 3 Bacterial Diseases, Edward Arnold, London, pp 365-379	Importance, anthrax in animals, persistence in the environment, anthrax in man, bacteriology, pathology, prevention, treatment.
Turnbull PCB (1991). Anthrax vaccines: past, present and future. Vaccine.. 9: 8, 533-539.	
Turnbull PCB (1998). Anthrax. In Zoonoses: biology, clinical practice and public health control, edited by SR Palmer, Lord Soulsby, DIH Simpson, Oxford	

University Press, Oxford, UK:. 3-16.	
Turnbull PCB (1998). Guidelines for the Surveillance and Control of Anthrax in Humans and Animals, 3rd Edition, World Health Organization, Geneva. WHO/EMC/ZDL/98.6	http://www.who.int/emcdocuments/zoonoses/docs/whoemczdi986.html
Turnbull PCB (1999). Definitive identification of *Bacillus anthracis*: a review. J Appl Microbiol 87: 237-240	
Turnbull PCB (2000) Current status of immunization against anthrax: old vaccines may be here to stay for a while. Curr Opin Infect Dis. 13(2):113-120.	Arjemptur Technology Ltd, Porton Down Science Park, Salisbury, UK. Anthrax vaccination has become a 'hot' topic. On the one hand, fears that Iraq holds secret caches of anthrax-based weaponry, that other countries may be developing or may have developed similar devices, or that hard-line groups may make their own anthrax-based devices for bioterrorist attacks have focused official attention on the need for means of protection, principally, though, for the military. On the other hand, the unsolved issues of the Gulf War illnesses have left elements of doubt in the minds of some as to the possible role of anthrax (among other) vaccines in this syndrome, and have drawn attention to the shortage of pre-clinical, clinical, pharmacological and safety data on the existing UK and US anthrax vaccines. In the middle are those hotly debating the US and Canadian policies of mandatory anthrax immunization for military personnel or, in the case of the UK policy of voluntary immunization, simply voting with their feet. Compounding matters have been the publicized failures of the US vaccine production facility and the less publicized UK problems of supply. Meanwhile, those in genuine at-risk occupations are left unsure whether, if they can get the vaccine at all, they really want it. Despite two decades of elegant science aimed at formulating alternative vaccines to overcome all the problems of efficacy, safety and supply, such an alternative is at least five years away, and the current status is that we must live with the old vaccines or not vaccinate.
Turnbull PCB (2000). Anthrax. Manual of standards for diagnostic tests and vaccines. List A and B diseases of mammals, birds and bees. Office International des Epizooties, Paris, France:. Ed.4, 233-244.	
Turnbull PCB, Bell RHV, Saigawa K, Munyenyembe FEC, Mulenga CK, Makala LHC (1991). Anthrax in wildlife in the Luangwa Valley, Zambia. Veterinary Record 128: 399–403.	Fate of spores and vegetative organisms added to water. Serology in nonvaccinated humans
Turnbull PCB, Broster MG, Carman JA, Manchee RJ, Melling J. (1986). Development of antibodies to protective antigen and lethal factor components of anthrax toxin in humans and guinea pigs	

and their relevance to protective immunity. Infection and Immunity.. 52: 2, 356-363.	
Turnbull PCB, Carman JA, Lindeque PM, Joubert F, Hubschle OJB, Snoeyenbos GH. (1989). Further progress in understanding anthrax in the Etosha National Park. Madoqua. 16: 2, 93-104.	Of 81 samples of water from pools, mud and soil collected from sites not connected with anthrax only one water sample contained *Bacillus anthracis*. The organism was isolated from 5 of 11 soil samples collected from sites where carcasses of animals known to have or suspected of having anthrax had lain. B. anthracis was also isolated from faeces of vultures and jackals, but not from 6 randomly collected bone samples. Six of 7 wildebeest, zebras and springbok found dying in the park were positive for anthrax. All of 7 lions tested had positive titres for anthrax, but 3 elephants, 2 zebra and 2 of 3 rhinos were negative (the other was thought to have been previously vaccinated). In laboratory tests vegetative forms of B. anthracis inoculated into water samples declined rapidly in number and the spores showed no sign of germination. It is suggested that water holes are not sites of germination and multiplication of B. anthracis.
Turnbull PCB, Doganay M, Lindeque PM, Aygen B, McLaughlin J. (1992). Serology and anthrax in humans, livestock and Etosha National Park wildlife. Epidemiology and Infection.. 108: 2, 299-313.	Results are presented from a number of epidemiological studies using enzyme immunoassays (EIA) based on the purified anthrax toxin antigens, protective antigen, lethal factor and oedema factor. Studies on sera from a group of 62 human anthrax patients in Turkey and from cattle in Britain following two unrelated outbreaks of anthrax show that EIA using protective antigen can be a useful diagnostic aid and will detect subclinical infections in appropriate circumstances. A serological survey on wildlife in the Etosha National Park, Namibia, where anthrax is endemic, showed that naturally acquired anthrax-specific antibodies are rare in herbivores but common in carnivores; in carnivores, titres appear to reflect the prevalence of anthrax in their ranges. Problems, as yet unresolved, were encountered in studies on sera from pigs following an outbreak of anthrax on a farm in Wales. Clinical details, including treatment, of the human and one of the bovine outbreaks are summarized and discussed in relation to the serological findings. 13/14 cattle with suspected anthrax recovered after single treatment with penicillin, streptomycin and flunixin. Vaccinated cattle had titres of 512 and 1024 (anti PA titres). EIA-detected Abs to PA reported in workers involved in production of Sterne vaccine.
Turnbull PCB, Hugh-Jones ME, Cosivi O. (1999). World Health Organization activities on anthrax surveillance and control. J Appl Microbiol 87: 318-320	
Turnbull PCB, Hutson RA, Ward MJ, Jones MN, Quinn CP, Finnie NJ, et al. (1992). *Bacillus anthracis* but not always anthrax. J Appl Bacteriol 72: 21–28.	[Hoffmaster et al 2002]
Turnbull PCB, Leppla SH, Broster MG et al: (1988). Antibodies to anthrax toxin in humans and guinea pigs and their relevance to protective immunity. Med Microbiol Immunol 177:293-303	Brachmann et al 2004 (#115)
Turnbull PCB, Lindeque PM, Roux J le, Bennett AM, Parks SR (1998). Airborne movement of anthrax spores from	Studies on pulmonary anthrax in animals (Druett et al 1953) reveal that at sizes above 5mcg particles face increasing difficulty in reaching the alveoli. It seems probable that few if any spores forming in soil from vegetative forms shed by dead animals remain free and

carcass sites in the Etosha National Park, Namibia. J Appl Microbiol 84: 667-676	unattached to soil particles. Windborne spores contribute little if anything to the spread of disease.
Turnbull PCB, Quinn CP, Henderson I. (2002). *Bacillus anthracis* and other Bacillus species. Molecular medical microbiology: Academic Press, London, UK:. 2011-2031.	This chapter discusses the taxonomy, identification, detection and diagnosis, virulence factors and genetic factors of virulence of Bacillus. Vaccination against B. anthracis is also discussed.
Turnbull PCB, Quinn CP, Hewson R, Stockbridge MC, Melling J. (1990). Protection conferred by microbially -- supplemented UK and purified PA vaccines. Salisbury Medical Bulletin, Supplement 68: 89-91	PA induces effective protective immunity, but the degree of this immunity in an individual cannot be determined from a serological anti-PA titre.
Turnbull PCB, Sirianni NM, LeBron CI, Samaan MN, Sutton FN, Reyes AE, Peruski LF Jr. (2004). MICs of selected antibiotics for *Bacillus anthracis*, Bacillus cereus, Bacillus thuringiensis, and Bacillus mycoides from a range of clinical and environmental sources as determined by the Etest. J Clin Microbiol. 42(8):3626-34.	Biological Defense Research Directorate, Naval Medical Research Center, Silver Spring, MD 20910-7500, USA. turnbullp@nmrc.navy.mil This paper presents Etest determinations of MICs of selected antimicrobial agents for 76 isolates of *Bacillus anthracis* chosen for their diverse histories and 67, 12, and 4 cultures, respectively, of its close relatives B. cereus, B. thuringiensis, and B. mycoides derived from a range of clinical and environmental sources. NCCLS breakpoints are now available for B. anthracis and ciprofloxacin, penicillin, and tetracycline; based on these breakpoints, the B. anthracis isolates were all fully susceptible to ciprofloxacin and tetracycline, and all except four cultures, three of which had a known history of penicillin resistance and were thought to originate from the same original parent, were susceptible to penicillin. Based on NCCLS interpretive standards for gram-positive and/or aerobic bacteria, all cultures were susceptible to amoxicillin-clavulanic acid and gentamicin and 99% (one with intermediate sensitivity) of cultures were susceptible to vancomycin. No group trends were apparent among the different categories of B. cereus (isolates from food poisoning incidents and nongastrointestinal infections and food and environmental specimens not associated with illness). Differences between B. anthracis and the other species were as expected for amoxicillin and penicillin, with all B. anthracis cultures, apart from the four referred to above, being susceptible versus high proportions of resistant isolates for the other three species. Four of the B. cereus and one of the B. thuringiensis cultures were resistant to tetracycline and a further six B. cereus and one B. thuringiensis cultures fell into the intermediate category. There was a slightly higher resistance to azithromycin among the B. anthracis strains than for the other species. The proportion of B. anthracis strains fully susceptible to erythromycin was also substantially lower than for the other species, although just a single B. cereus strain was fully resistant. The Etest compared favorably with agar dilution in a subsidiary test set up to test the readings, and it compared with other published studies utilizing a variety of test methods.
Turnbull PCB, Tindall BW, Coetzee JD, Conradie CM, Bull RL, Lindeque PM, Huebschle OJ. (2004). Vaccine-induced protection against anthrax in cheetah (Acinonyx jubatus) and black rhinoceros (Diceros bicornis). Vaccine. 22(25-	Arjemptur Technology, Science Park, Dstl, Porton Down, Salisbury SP4 0JQ, UK. peterturnbull@tesco.net Institution of a policy of vaccination in endangered species with a vaccine not previously administered to it cannot be undertaken lightly. This applies even more in the case of cheetah (Acinonyx jubatus) with their unusually monomorphic gene pool and the potential restrictions this places on their immune responses. However, the recently observed mortalities from anthrax in these animals in the Etosha National Park, Namibia, made it imperative to evaluate vaccination. Black rhinoceros (Diceros bicornis), another endangered species in the park, have been vaccinated for

26):3340-7.	over three decades but the effectiveness of this has never been evaluated. Passive protection tests in A/J mice using sera from 12 cheetahs together with enzyme immunoassay indicated that cheetah are able to mount seemingly normal primary and secondary humoral immune responses to the Sterne 34F2 live spore livestock vaccine. Overall protection rates in mice injected with the sera rose and fell in concert with rises and declines in antibody titres, although fine analysis showed that the correlation between titre and protection was complex. Once a high level of protection (96% of mice 1 month after a second booster in the cheetahs) had been achieved, the duration of substantial protection appeared good (60% of the mice 5 months after the second booster). Protection conferred on mice by sera from three of four vaccinated rhino was almost complete, but, obscurely, none of the mice receiving serum from the fourth rhino were protected. Sera from three park lions with naturally acquired high antibody titres, included as controls, also conferred high levels of protection. For the purposes of wildlife management, the conclusions were that vaccination of cheetah with the standard animal anthrax vaccine causes no observable ill effect in the animals and does appear to confer protective immunity. At least one well-separated booster does appear to be desirable. Vaccination of rhino also appears to be justified from the limited data obtained.
Turner AJ, Galvin JW, Rubira RJ, Condron RJ, Bradley T. (1999b). Experiences with vaccination and epidemiological investigations on an anthrax outbreak in Australia in 1997. Journal of Applied Microbiology.. 87: 2, 294-297.	Between January and February 1997, there was a severe outbreak of anthrax on 83 properties in north-central Victoria, Australia. Vaccination was used as a major tool to control the outbreak by establishing a vaccination buffer zone 30 km by 20 km. In all, 78 649 cattle in 457 herds were vaccinated in a three week programme. In the face of the outbreak, there was a delay before vaccination was able to stop deaths. In the 10 days following vaccination 144 cases of confirmed anthrax occurred and 38 cases occurred more than 10 days after vaccination. When all cattle on properties at-risk were revaccinated in October and early November 1997, there were only two confirmed cases of anthrax in vaccinated seven and nine month old calves in the following anthrax season. Investigations into the epidemiology of the outbreak were unable to establish a single major association for the spread of the disease by flies, biting insects, carrion scavengers, wind, manufactured feed, milk factory tanker routes, veterinary visits, animal treatments, movements of personnel between farms or burning of carcasses. The weather conditions in the outbreak area were part of a long dry spell with periods of high daily and night temperatures, continuing high humidity over the period and higher than normal soil temperatures. It is possible that extensive earth works in the district involving irrigated pasture renovation and water channel and drainage renovation could have disturbed old anthrax graves. These works may have released spores that were dispersed in the preceding wet winter across poorly drained areas that formed the axis for the outbreak. The earth moving renovations establishing irrigation in the area were conducted in the late 1890s, and before the occurrence of anthrax outbreaks were recorded. The axis of the outbreak was the major stock route for cattle and sheep moving from southern Victoria to northern Victoria and southern New South Wales, and undoubtedly there would have been extensive anthrax outbreaks before vaccine became available in the 1890s. In respect of other outbreaks, the events in Victoria most resembled outbreaks of anthrax recorded in the USA in the 1950s, 1960s and 1970s.
Turner AJ, Galvin JW, Rubira RJ, Miller GT. (1999a). Anthrax explodes in an Australian summer. Journal of Applied Microbiology.. 87: 2, 196-199.	Anthrax occurred on 83 properties in an area of north central Victoria between 26 January and 26 March in the summer of 1997. Anthrax had not been recorded in the outbreak area since records were started in 1914, although anthrax did occur in the general area in the 1880s to 1890s. Standard Australian control measures were applied to the properties, including quarantine, tracing movements of animals on and off affected properties, secure disposal of carcasses by burning, enhanced surveillance of stock generally in the area and the use of local disaster control procedures including an alert of health authorities. As affected property numbers began to increase dramatically from 8 February, it was decided to use blanket area vaccination to control the disease. By 26 February, the epidemic curve had returned to the base line and a buffer vaccination zone of 457 farms holding 78 649 cattle was formed by early March 1997. Between 26 January and 26 March when the outbreak was declared over, 202 cattle and 4 sheep were confirmed to have died of

	anthrax. Between 27 March and early November a further 26 cattle were confirmed as dying due to anthrax and 14 of these had not had previous vaccination, including four young calves and one horse. One new property within the vaccination buffer zone had an anthrax case in a cow in early November 1997. By mid-November 1997, all previously infected and all neighbouring properties within 1 km were compulsorily re-vaccinated, as were all calves when two months of age and all introduced cattle. In 1998, only two confirmed cases of anthrax were diagnosed; both were vaccinated calves on farms which had had multiple cases during the outbreak. The public reaction and attention fuelled by unprecedented media attention led to intense international scrutiny from countries where anthrax is a particular zoonotic problem. Very strong representations had to be made about the safety of livestock and livestock products that came from Victoria. This event has demonstrated that there is a need to review OIE and other requirements and recommendations covering anthrax where strict restrictions are placed on livestock and livestock products to protect livestock and human populations against anthrax infection.
Turner M. (1980) Anthrax in humans in Zimbabwe. Cent Afr J Med 26: 160-161	
Uchida I, Hashimoto K, Terakado N. (1986). Virulence and immunogenicity in experimental animals of *Bacillus anthracis* strains harbouring or lacking 110 MDa and 60 MDa plasmids. Journal of General Microbiology.. 132: 2, 557-559.	The virulence for mice of B. anthracis strains cured of the 110 MDa or the 60 MDa plasmid was less than a hundredth of the parental strains harbouring these plasmids. Guinea-pigs immunized with plasmid-free derivatives of the non-encapsulated vaccine strain 34F2 showed no resistance to challenge with strain 17JB, which harbours both 110 MDa and 60 MDa plasmids, suggesting that the derivative strains had lost their immunogenicity.
UK Department of Health (1995). Milk Supplies in Relation to Anthrax in Dairy Herds/Flocks (England and Wales), United Kingdom Department of Health. Circular PL/CO (95)3.	
Urguev KR, Bakulov IA, Nazhalov MA, Nuratinov RA, Gavrilov VA, Seliverstov VV. (1999). Development of a mixed vaccine against anthrax and clostridiosis of sheep. Russian Agricultural Sciences. . 8, 42-46.	A mixed vaccine has been developed against anthrax and clostridiosis of sheep, and its immunizing properties were studied. The vaccine ensured immunity for over nine months against clostridiosis and over a year against anthrax.
Urguev KR, Kirillov LV, Romanov GI, Storozhev LI, Manichev AA. (1989). Combined live vaccine against anthrax and blackleg. Veterinariya, Moscow.. 4, 29-30.	The combined vaccine was prepared from attenuated strain "2/14" of Clostridium chauvoei and strain "STI-1" of *Bacillus anthracis*. It was tested on 5360 cattle in 1984, 158 000 in 1986 and 218 000 in 1987 with satisfactory results. Challenge infection experiments were conducted on calves and sheep.
Vaissaire J, Mock M, Doujet C le; Levy M. (2001). Anthrax. Epidemiologic study of the disease in France. Medecine et	A retrospective study of anthrax cases in animals, which occurred in France, between 1980 and 2000, showed that a total of 114 anthrax outbreaks were recorded during the said period, 44 of which occurred during 1997-2000. These indicate an increase in animal cases of anthrax. Human cases and/or contaminations were significantly associated with

Maladies Infectieuses. 31: Supplement 2, 257s-271s.	the animal disease, except for 3 cases. Cutaneous anthrax was the most common form noted in human cases. Thus, animal vaccinations and veterinary supervision should again be recommended. Also, increasing one's vigilance for animal cases and human contamination should be emphasized.
Vaissaire J, Mock M, Patra G, Valognes A, Grenouillat D, Pion I, Gauthier D, Ricart J, Doujet C, Weber M, Pedaille F, Patty R, Prudhomme C, Game Y. (1997). Outbreaks of anthrax in France in 1997 in different animals and man. Applications of new diagnostic techniques. Bulletin de l'Academie Veterinaire de France.. 70: 4, 445-456.	Two important outbreaks of anthrax zoonoses in the French Pyrenees and the French Alps in 1997 are described. Most cases occurred in cows during the summer and autumn. 80 animals died. There were 3 non-fatal cases in humans and one in a dog. The diagnosis of anthrax was confirmed by bacteriological and molecular methods. The multiplex polymerase chain reaction assay was used to characterize B. anthracis strains from animal samples. It appears that a penicillin-resistant B. anthracis strain was isolated, for the first time in France.
Van der Hoeden J (1964). Anthrax. In Zoonoses, edited by J van der Hoeden, Elsevier Publishing Company, Amsterdam pp 202-223	
Van Ness G, Stein CD. (1956). Soils of the United States favorable for anthrax. J Am Vet Med Assoc January 1, ,7-9.	
Van Ness GB (1972) Ecology of anthrax. Science 172: 1303-1307	'In the Oklamoma outbreak of 1957 60% of the herds studies lost only one animal'
Van Ness GB, Plotkin SA, Huffaker RH, Evans WG. (1959) The Okalahoma-Kansas anthrax epizootic of 1957. Journal of the American Veterinary Medical Association 134: 125–129	First report of widespread use of Sterne vaccine. Biting flies not considered likely transmitters of anthrax Mortalities in 127 vaccinated herds decreased 8 days after vaccination.
Van Ness GB. (1969). Anthrax incubator areas. J Am Vet Med Assoc 155: 503	
Varga J. (2001). Importance of milk in animal and human health. Allattenyesztes es Takarmanyozas. 50: 5, 412-421.	Emphasising the immunobiological importance of colostrum, the author summarises the most important rules of milk production, processing and commerce, and provides an overview of those infections or diseases the causative agents of which are frequently transmitted by milk and against which both animals and humans are susceptible (zoonoses). Apart from its nutritive value, colostrum provides the sole source of immunoglobins for most domestic animals. As immunoglobulins in unbroken form can be absorbed in cattle practically only for 24 hours after birth, free access to colostrum is essential for the newly born animals, minimally during the first two days of life. Rules of milk production and processing must be rigorously observed in order to obtain milk of good quality in respect of both physical, chemical and microbiological terms. All those bacteria and viruses which are able to cause systemic infections or are present only in the udder can be shed in the milk for shorter or longer periods, and many of these agents may give rise to infection in humans. Anthrax, listeriosis, bovine tuberculosis, Salmonella and Campylobacter infections, brucellosis, leptospiroses, chlamydiosis, Q-fever and tick borne encephalitis are the most important zoonotic diseases in this country which are or

	can be transmitted frequently by milk. Rigorous observation of the rules of milk production helps avoid contamination and heat treatment can destroy all pathogenic agents but drinking of raw milk means a certain risk.
Venkatesh S, Memsih ZA (2003). Bioterrorism – a new challenge for public health. Int J Antimicrob Agents 21: 200-206	
Verner OM, Sinyak KM, Volkova VP. (1988). Composition of fatty acids of *Bacillus anthracis* and other soil bacilli. Biology Bulletin of the Academy of Sciences of the USSR.. 15: 1, 25-30.	The composition of fatty acids of cellular lipids of *Bacillus anthracis* STI vaccine strain, and a number of other bacilli living in soil (B. cereus, B. subtilis, B. mesentericus, B. mycoides, B. megaterium, B. anthracoides, B. pseudoanthracis) was studied. It was shown that almost all bacilli studied differed from the anthrax pathogen by having a substantial content of C18 fatty acids and a lower content of pentadecanoic acid (C15:0). Comparative analysis of fatty acid profiles of B. anthracis vaccine strain STI, and B. cereus during cultivation on media of differing composiiton showed high lability of quantitative content of a number of fatty acids in both bacilli. More stable composition of fatty acids distinguished B. anthracis. The possibility is discussed of using fatty acid characteristics to differentiate the anthrax pathogen from other taxonomically similar bacilli which live in soil, and also to identify relationships between individual taxons.
Vibha Chauhan; Aparna Singh; Waheed, S. M, Samer Singh; Rakesh Bhatnagar. (2001). Constitutive expression of protective antigen gene of *Bacillus anthracis* in Escherichia coli. Biochemical and Biophysical Research Communications.. 283: 2, 308-315.	The constitutive expression of the protective antigen (PA) gene of *Bacillus anthracis*, using Escherichia coli DH5 alpha strain was reported. A high-density batch culture technique was also used to scale-up the expression and yield of recombinant PA. It was observed that PA protein was expressed simultaneously with the culture growth and there was no impairment of culture growth due to constitutive expression of the PA protein. The expressed recombinant protein was found to be localized inside inclusion bodies in the cell. The plasmid was stably present inside the cells and expressing the protein to normal levels. For the biomass scale-up, there was about a three- to fourfold increase. Approximately 125 mg of the purified protein from 1 litre culture broth was obtained upon purification of the recombinant PA protein. Macrophage lysis assay resulted in the recombinant PA with lethal factor fully lysing macrophage cells; its biological activity was similar to the native PA. The results demonstrate that high cell densities are obtainable for this expression system, with concomitant higher recombinant protein expression. A large-scale production of PA protein based anthrax vaccine is made possible due to the findings of this work.
Vitale G, Pellizzari R, Recchi C, Napolitani G, Mock M, Montecucco C. (1999). Anthrax lethal factor cleaves the N-terminus of MAPKKS and induces tyrosine/threonine phosphorylation of MAPKS in cultured macrophages. J Appl Microbiol 87: 288	
Vodkin MH, Leppla SH. (1983). Cloning of the protective antigen gene of *Bacillus anthracis*. Cell 34(2): 693-697	The tripartite protein toxin of *Bacillus anthracis* consists of protective antigen (PA), edema factor (EF), and lethal factor (LF). As a first step in developing a more efficacious anthrax vaccine, recombinant plasmids containing the PA gene have been isolated. A library was constructed in the E. coli vector pBR322 from Bam HI-generated fragments of the anthrax plasmid, pBA1. Two clones producing PA were identified by screening lysates with ELISA (enzyme-linked immunosorbent assay). Western blots revealed a full-size PA protein in the recombinant E. coli, and a cell elongation assay demonstrated biological activity. Both positive clones had a 6 kb insert of DNA, which mapped in the Bam HI site of the vector. The two inserts are the same except that they lie in opposite orientations with respect to the vector. Thus PA is encoded by the plasmid pBA1.

Vogel FR and Powell MF. (1995). Compendium of vaccine adjuvants and excipients. In Powell MF and Newman MJ (eds): Vaccine design: the subunit and adjuvant approach. New York, Plenum Press, , p. 1464	
Vylchev V, Siromashkova M, Mircheva I. (1976). Standardization of a protective anthrax preparation. Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii.. 4, 53-56.	
Wade BH, Wright GG, Hewlett EL, Leppla SH, Mandell GL (1985). Anthrax toxin components stimulate chemotaxis of human polymorphonuclear neutrophils. Proc Soc Exp Biol Med 179: 159–62.	[During et al 2001, 13]
Wade N. (1980). Death at Sverdlovsk: a critical diagnosis. [An anthrax outbreak.]. Science. 209: 4464, 1501-1502.	
Waldrup KA, Conger TH. (2002). Maintaining a vigilance for foreign animal diseases. Vet Clin North Am Food Anim Pract 18(3): 379-87	Texas Animal Health Commission, 2105 Kramer Lane, Austin, TX 78758, USA. The incursion of foot-and-mouth disease (FMD) into the United Kingdom in February 2001 served as a wakeup call for North American agriculture. As the livestock health crisis in the United Kingdom progressed, it became increasingly evident that the United States, Canada, and Mexico were also susceptible to an incursion of a foreign animal disease. The terrorist attacks of September 11, 2001, and the subsequent anthrax mailings reaffirmed the fact that the United States is vulnerable to an infectious assault, regardless of whether it is intentional or accidental.
Walley T (1883). Anthrax. Lancet 121(3100): 169	Anthrax outbreak in a flock of 40 ewes. Anthrax lesion found in foetuses of three ewes which died.
Walton JR. (1992). Anthrax. *In* Diseases of swine, edited by AD Leman, BE Straw, WL Mengeling, S D'Allaire and DJ Taylor, Iowa State University Press, Ames, Iowa 50010, USA:. 7th Edition, 409-413.	
Wang Jin; Song YaJun; Guo ZhaoBiao; Yang RuiFu. (2002). Duplex PCR for the direct detection of anthrax spores from artificially contaminated soil. Chinese Journal of Zoonoses.. 18: 6, 52-54, 72.	A study was conducted to establish a duplex polymerase chain reaction, based on genes in 2 virulence-related plasmids to detect anthrax spores from artificially contaminated soil. Based on gene encoding oedema factor on plasmid pX01 and capsule gene on plasmid pX02, two sets of primers were designed to establish duplex PCR. The sensitivity and specificity of the duplex PCR were evaluated and the method were used to directly detect anthrax spores from soil contaminated artificially by spores of *Bacillus anthracis* vaccine strain A16.R. The ratio and the concentration of the primers used were the most important factors than others, such as Mg2+ and dNTP concentration, PCR cycling parameters, affecting the results of duplex PCR. The sensitivity of duplex PCR was 10 times lower than that of single-

	gene based PCR. Both duplex and single gene-based PCR could detect 103 spores/0.25 g soil. Results show that duplex PCR is a powerful alternative technique to directly detect anthrax spores from suspected soil.
Wang JY, Roehrl MH (2005). Anthrax vaccine design: strategies to achieve comprehensive protection against spores, bacillus and toxin. Medical Immunology 4: 4	
Wang TT, Fellows PF, Leighton TJ, Lucas AH (2004). Induction of opsonic antibodies to the Q-D-glutamic acid capsule of *Bacillus anthracis* by immunization with a synthetic peptide-carrier protein conjugate. FEMS Immunology and Medical Microbiology 40: 231-237	The capsule of *Bacillus anthracis*, a polymer of Q-D-glutamic acid, functions as a virulence determinant and is a poor immunogen. In this study we show that antibodies reactive with the B.anthracis capsule can be elicited in mice by immunization with a conjugate consisting of a synthetic Q-D-glutamic acid nonamer peptide (Q-D-glu9) covalently coupled to keyhole limpet hemocyanin. The serum response to Q-Dglu9 was comprised primarily of IgG antibodies that recognized an epitope requiring a minimum of four Q-linked D-glutamic acid residues. Antibodies to (Q-D-glu9) bound to the surface of encapsulated B.anthracis cells and mediated opsonophagoctosis. These findings suggest that anti-capsular antibodies could mediate the clearance of vegetative B.anthracis cells in vivo. Thus, inclusion of an immunogenic capsular component as well as protective antigen in new anthrax vaccines would generate immune responses targeting both the bacteremic and toxigenic aspects of anthrax infection and thus may increase protective efficacy.
Watson A, Keir D. (1994) Information on which to base assessments of risk from environments contaminated with anthrax spores. Epidemiol Infect. 113(3): 479-490	Environmental Risk Assessment Department, AEA Technology Consultancy Services, Warrington, Cheshire. Although there has been a considerable amount of research conducted into *Bacillus anthracis*, the causative agent of anthrax, the data are widely disseminated in the scientific literature and are therefore not always easy to assimilate. In view of continuing concern about potential anthrax contamination in environmental materials and sites, this review brings together the currently available information relating to the health hazards from B. anthracis. The relevance of the available information for risk assessment purposes is assessed.
Watson J, Koya V, Leppla SH, Daniell H. (2004). Expression of *Bacillus anthracis* protective antigen in transgenic chloroplasts of tobacco, a non-food/feed crop. Vaccine. 22(31-32):4374-84.	Department of Molecular Biology and Microbiology, University of Central Florida, Biomolecular Science Building #20, Room 336, Orlando, FL 32816-2364, USA. The Centers for Disease Control (CDC) lists *Bacillus anthracis* as a category A agent and estimates the cost of an anthrax attack to exceed US$ 26 billion per 100,000 exposed individuals. Concerns regarding anthrax vaccine purity, a requirement for multiple injections, and a limited supply of the protective antigen (PA), underscore the urgent need for an improved vaccine. Therefore, the 83 kDa immunogenic *Bacillus anthracis* protective antigen was expressed in transgenic tobacco chloroplasts. The PA gene (pag) was cloned into a chloroplast vector along with the psbA regulatory signals to enhance translation. Chloroplast integration of the transgenes was confirmed by PCR and Southern blot analyses. Crude plant extracts contained up to 2.5 mg full length PA/g of fresh leaf tissue and this showed exceptional stability for several months in stored leaves or crude extracts. Maximum levels of expression were observed in mature leaves under continuous illumination. Co-expression of the ORF2 chaperonin from Bacillus thuringiensis did not increase PA accumulation or induce folding into cuboidal crystals in transgenic chloroplasts. Trypsin, chymotrypsin and furin proteolytic cleavage sites present in PA were protected in transgenic chloroplasts because only full length PA 83 was observed without any degradation products. Both CHAPS and SDS detergents extracted PA with equal efficiency and PA was observed in the soluble fraction. Chloroplast-derived PA was functionally active in lysing mouse macrophages when combined with lethal factor (LF). Crude leaf extracts contained up to 25 microg functional PA/ml. With an average yield of 172 mg of PA per plant using an experimental transgenic cultivar grown in a greenhouse, 400 million doses of vaccine (free of contaminants) could be produced per acre, a yield that could be further enhanced 18-

	fold using a commercial cultivar in the field.
Weber DJ, Sickbert-Bennett E, Gergen MF, Rutala WA (2003). Efficacy of selected hand hygiene agents used to remove Bacillus atrophaeus (a surrogate for *Bacillus anthracis*) from contaminated hands. JAMA 289: 1274-1277	Soap and water, 2% chlorhexidine gluconate, waterless 61% ethyl alcohol, hypochlorite releasing microfibre towel. Spore counts reduced with all washes except waterless 61% ethyl alcohol.
Webster A (1973). Inhibiting effect of antibiotics on anthrax vaccination. Aust Vet J 49(11): 545	Study in guinea pigs
Wei Z, Schnupf P, Poussin MA, Zenewicz LA, Shen H, Goldfine H (2005). Characterization of Listeria monocytogenes Expressing Anthrolysin O and Phosphatidylinositol-Specific Phospholipase C from Bacillus anthracis. Infect Immun 73: 6639-6646.	Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6076, :goldfinh@mail.med.upenn.edu Two virulence factors of Listeria monocytogenes, listeriolysin O (LLO) and phosphatidylinositol-specific phospholipase C (PI-PLC), mediate escape of this pathogen from the phagocytic vacuole of macrophages, thereby allowing the bacterium access to the host cell cytosol for growth and spread to neighboring cells. We characterized their orthologs from Bacillus anthracis by expressing them in L. monocytogenes and characterizing their contribution to bacterial intracellular growth and cell-to-cell spread. We generated a series of L. monocytogenes strains expressing B. anthracis anthrolysin O (ALO) and PI-PLC in place of LLO and L. monocytogenes PI-PLC, respectively. We found that ALO was active at both acidic and neutral pH and could functionally replace LLO in mediating escape from a primary vacuole; however, ALO exerted a toxic effect on the host cell by damaging the plasma membrane. B. anthracis PI-PLC, unlike the L. monocytogenes ortholog, had high activity on glycosylphosphatidylinositol-anchored proteins. L. monocytogenes expressing B. anthracis PI-PLC showed significantly decreased efficiencies of escape from a phagosome and in cell-to-cell spread. We further compared the level of cytotoxicity to host cells by using mutant strains expressing ALO in combination either with L. monocytogenes PI-PLC or with B. anthracis PI-PLC. The results demonstrated that the mutant strain expressing the combination of ALO and B. anthracis PI-PLC caused less damage to host cells than the strain expressing ALO and L. monocytogenes PI-PLC. The present study indicates that LLO and L. monocytogenes PI-PLC has adapted for L. monocytogenes intracellular growth and virulence and suggests that ALO and B. anthracis PI-PLC may have a role in B. anthracis pathogenesis.
Wein LM, Liu Y, Leighton TJ (2005). HEPA/vaccine plan for indoor anthrax remediation. Emerg Infect Dis 11: 69-76	HEPA vacuuming, HEPA air cleaners, vaccination of reoccupants. “a safe, effective, singl-dose vaccine would have a profound impact onmitigating the undesirable circumstances of this scenario.”
Welkos S, Friedlander A, Weeks S, Little S, Mendelson I. (2002). In-vitro characterisation of the phagocytosis and fate of anthrax spores in macrophages and the effects of anti-PA antibody. J Med Microbiol 51(10): 821-831	susan.welkos@amedd.army.mil Antibodies (Abs) to the protective antigen (PA) component of the anthrax toxins have anti-spore as well as anti-toxin activities. Anti-PA antisera and purified anti-PA Abs enhance the phagocytosis by murine-derived macrophages (MQs) of spores of the Ames and Sterne strains and retard the germination of extracellular spores in vitro. The fate after phagocytosis of untreated and anti-PA-treated spores was further studied in culture medium that supported phagocytosis without stimulating spore germination (Dulbecco's minimal essential medium with horse serum 10%). The spores germinated within cells of primary peritoneal murine MQs (C3H/HeN) and MQs of the RAW264.7 MQ-like cell line; germination was associated with a rapid decline in spore viability. Exposure of MQs to inhibitors of phago-endosomal acidification (bafilomycin A and chloroquine) reduced the efficiency of MQ killing and allowed outgrowth and

	replication of the organisms. Treatment of spores with anti-PA Abs stimulated their phagocytosis and was associated with enhanced MQ killing of the spores. The enhanced killing of spores correlated with the greater extent of germination of anti-PA-treated spores after phagocytosis. A PA null mutant of the Ames strain exhibited none of the effects associated with anti-PA Ab treatment ofthe parental strain. Thus, the anti-PA Ab-specific immunity induced by vaccines has anti-spore activities and its role in impeding the early stages of infection with Bacillusanthracis needs to be assessed.
Welkos SL (1991). Plasmid-associated virulence factors of non-toxigenic (pX01-) Bacillus anthracis. Microb Pathog 10(3): 183-198	Division of Bacteriology, United States Army Medical Research Institute of Infectious Diseases, Frederick, MD 21702-5011. The anthrax toxins and capsule, encoded by plasmids pX01 and pX02, respectively, are the only known virulence factors of Bacillus anthracis and are considered essential for full virulence. Some B. anthracis strains cured of pX01, such as delta Ames-1, remained virulent for mice. The virulence was partially mediated by pX02, as determined by phage transduction. pX02 plasmids from the delta Ames-1 and Pasteur strains were mutagenized with transposon Tn917 to identify loci associated with virulence. The capsule phenotype, virulence and pX02 restriction pattern of the insertion mutants were characterized. Two mutants that produced no detectable capsule were avirulent. One had a deletion of more than 20 kb, which included the structural genes required for capsule synthesis (cap); the second had an insertion outside of cap. Two mutants with reduced encapsulation had insertions at different sites outside cap and were less virulent, whereas one that was normally encapsulated, but had a high rate of pX02 curing, was unaltered in virulence. Mutants that produced greater amounts of capsule than the parental strain were more virulent, and a few that produced wild-type levels of capsule were less virulent.
Welkos SL, Becker D, Friedlander AF, Trotter R. (1990). Pathogenesis and host resistance to *Bacillus anthracis*: a mouse model. Salisbury Medical Bulletin, Supplement 68: 49-52	
Welkos SL, Becker D, Keener T. (1987). Pathogenesis, protective efficacy, and genetic control of resistance to *Bacillus anthracis* Sterne strain in mice. Abstracts of the Annual Meeting of the American Society for Microbiology.. 87: 40.	
Welkos SL, Cote CK, Rea KM, Gibbs PH. (2004). A microtiter fluorometric assay to detect the germination of Bacillus anthracis spores and the germination inhibitory effects of antibodies. J Microbiol Methods 56(2): 253-265	Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702, USA. susan.welkos@DET.AMEDD.army.mil Bacillus anthracis spore germination is usually detected in vitro by alterations in spore refractility, heat resistance, and stainability. We developed a more quantitative, sensitive, and semi-automated procedure for detecting germination by using a microtiter kinetic reader for fluorescence spectrophotometry. The procedure was based on the increase in fluorescence of spores with time during their incubation in germination medium containing a fluorescent nucleic acid-binding dye which stained germinated B. anthracis but not ungerminated (UG) spores. Spore germination in the presence of several germinants was characterized. Although L-alanine and inosine alone stimulated rapid germination in this assay, a medium containing optimal concentrations of L-alanine, adenosine, and casamino acids gave low

	background fluorescence, stimulated germination completely, and at a reasonable rate. Suspensions of heat-activated, UG spores of B. anthracis strain Ames were preincubated with antibodies (Abs) against whole spores to assess their effect on germination. Analyses of the germination data obtained revealed significant differences between spores pretreated with these Abs and those treated with non-immune sera or IgG. Germination inhibitory activity (GIA) was detected for several polyclonal rabbit anti-spore Ab preparations. These included anti-Ames strain spore antisera, IgG purified from the latter, and spore affinity-purified Abs from antisera elicited against four strains of B. anthracis. Abs elicited against UG as well as completely germinated Ames spores inhibited germination. Abs were ranked according to their GIA, and those specific for UG spores usually exhibited greater GIA. Direct binding to spores of these Abs was detected by an ELISA with whole un-germinated Ames spores. Although specific binding to spores by the anti-spore Abs was shown, their titers did not correlate with their GIA levels. Current efforts are focused on identifying the spore antigens recognized by the anti-spore Abs, characterizing the role of these targeted antigens in disease pathogenesis, and evaluating the ability of specific anti-spore Abs to protect against infection with B. anthracis.
Welkos SL, Friedlander AM. (1988). Comparative safety and efficacy against *Bacillus anthracis* of protective antigen and live vaccines in mice. Microbial Pathogenesis.. 5: 2, 127-139.	Mice of strains A/J, CBA/J and BALB/cJ were higly susceptible to lethal infection by the Vollum 1B strain of *Bacillus anthracis*. Those of the A/J strain were also much more susceptible than the other two strains to lethal infection by the Sterne vaccine (104-fold difference in LD50) but all mouse strains were resistant to the B. subtilis PA2 strain which carried a recombinant plasmid containing the part of the pXO1 plasmid of B. anthracis encoding the protective antigen. Vaccination with B. subtilis PA2 protected CBA/J or BALB/cJ mice against the virulent Vollum 1B strain more effectively than the Sterne vaccine strain which was only protective at doses <more or =>0.1 LD50. Cell-free protective antigen vaccine was not effective in protecting these or A/J mice against lethal infection and the latter were not protected by any of the other vaccines either. However, A/J mice could be protected against the Sterne strain by immunization with B. subtilis PA2 and the survivors of this regimen showed increased resistance to the fully virulent Vollum 1B strain. Passive antibodies to PA2 also protected A/J mice against the Sterne strain. However for immunity against fully virulent strains it would appear that factors additional to antibodies to B. anthracis protective antigen are required. This is consistent with observations by others on the variable efficacy of cell-free vaccines based on protective antigen in protecting guineapigs against a range of virulent strains. It is uncertain how closely the responses of guineapigs and mice to B. anthracis cell-free antigen reflect the development of protective immunity in man.
Welkos SL, Friedlander AM. (1988). Pathogenesis and genetic control of resistance to the Sterne strain of Bacillus anthracis. Microb Pathog 4(1): 53-69	Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD 21701-5011. The pathogenesis of lethal infection by the nonecapsulated, toxigenic Sterne strain of Bacillus anthracis and the genetic basis of resistance were characterized in mice. Lethal doses of Sterne spores produced disease in susceptible mice similar to that caused by toxigenic and encapsulated B. anthracis. At the inoculation site, the mice developed an edematous exudate with large concentrations of bacilli and toxin. In the susceptible A/J strain, lethal infection was accompanied by systemic invasion and serum anthrax toxin levels increased in parallel with systemic bacterial concentrations and with the mortality rate. Host resistance to Sterne infection was associated with the ability to synthesize the complement component 5 (C5). All Sterne-resistant mouse strains had a functional gene (Hc) encoding C5, whereas susceptible mice were deficient in C5. A/J mice could be passively protected from lethal challenge by C5-positive serum but not by serum from C5-negative congenic mice. Also resistance was linked to production of C5 in individual backcross (97%) and F2 (98%) mice. The distribution pattern for recombinant inbred mice was consistent with a major role in host resistance of Hc or a closely linked locus, although other genes probably contribute. This mouse model will be useful in characterizing the pathogenesis of anthrax and testing the safety and efficacy of new anthrax vaccines.
Welkos SL, Little S, Friedlander A, Fritz D,	Vaccines which are efficacious against anthrax, such as the human vaccine, Anthrax Vaccine Absorbed (AVA), contain

Fellows P. (2001). The role of antibodies to *Bacillus anthracis* and anthrax toxin components in inhibiting the early stages of infection by anthrax spores. Microbiology (Reading). 147: 6, 1677-1685.	the protective antigen (PA) component of the anthrax toxins as the major protective immunogen. Although AVA protects against inhalational anthrax, the immune responses to and role in protection of PA and possibly other antigens have yet to be fully elucidated. Sera from animals immunized with a toxin-producing, unencapsulated live vaccine strain of B. anthracis have been reported to have anti-spore activities associated with the antitoxin humoral response. The authors performed studies to determine whether anti-PA antibody (Ab)-containing preparations stimulated spore uptake by phagocytes and suppressed the germination of spores in vitro. AVA- and PA-immune sera from several species enhanced the phagocytosis by murine peritoneal macrophages of spores of the virulent Ames and the Sterne vaccine strains. Antitoxin Abs appeared to contribute significantly, although not solely, to the enhanced uptake. Rabbit antisera to PA purified from either Sterne or a PA-producing pX01-cured recombinant, affinity-purified anti-PA IgG, and monkey antisera to AVA were used to assess the role of anti-PA Abs. Rabbit anti-PA Abs promoted the uptake of spores of the PA-producing strains Sterne, Ames and RP42, a mutant of Sterne producing only PA, but not of the pX01- Delta Sterne-1 strain, Delta Ames strain, or RP4, a mutant of Sterne with deletions in the loci encoding PA and the oedema factor (EF) toxin component and producing only the lethal factor toxin component. Rabbit anti-PA and monkey anti-AVA Abs also significantly inhibited spore germination in vitro compared to preimmune serum or medium. Spore-associated proteins recognized by anti-PA Abs were detected by electron microscopy and confirmed by immunoblotting of spore coat extracts. Thus, the anti-PA Ab-specific immunity induced by AVA has anti-spore activity and might have a role in impeding the early stages of infection with B. anthracis spores.
Welkos SL, Lowe JR, Eden-McCutchan F, Vodkin M, Leppla SH, Schmidt JJ. (1988). Sequence and analysis of the DNA encoding protective antigen of Bacillus anthracis. Gene 69(2): 287-300	Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD 21701-5011. The nucleotide sequence of the protective antigen (PA) gene from Bacillus anthracis and the 5' and 3' flanking sequences were determined. PA is one of three proteins comprising anthrax toxin; and its nucleotide sequence is the first to be reported from B. anthracis. The open reading frame (ORF) is 2319 bp long, of which 2205 bp encode the 735 amino acids of the secreted protein. This region is preceded by 29 codons, which appear to encode a signal peptide having characteristics in common with those of other secreted proteins. A consensus TATAAT sequence was located at the putative -10 promoter site. A Shine-Dalgarno site similar to that found in genes of other Bacillus sp. was located 7 bp upstream from the ATG start codon. The codon usage for the PA gene reflected its high A + T (69%) base composition and differed from those of genes for bacterial proteins from most other sequences examined. The TAA translation stop codon was followed by an inverted repeat forming a potential termination signal. In addition, a 192-codon ORF of unknown significance, theoretically encoding a 21.6-kDa protein, preceded the 5' end of the PA gene.
Welkos SL, Trotter RW, Becker DM, Nelson GO. (1989). Resistance to the Sterne strain of B. anthracis: phagocytic cell responses of resistant and susceptible mice. Microb Pathog. 7(1): 15-35	Division of Bacteriology, United States Army Medical Research Institute of Infectious Diseases, Frederick, MD 21701-5011. Inflammatory responses were compared in vivo, and host phagocytic cell functions compared in vitro, of mice resistant (CBA/J) and susceptible (A/J) to lethal infection with the Sterne strain of Bacillus anthracis. Polymorphonuclear leukocyte (PMN) and macrophage responses at the initial site of infection were slower in A/J mice than in CBA/J mice. Whereas in A/J mice, the number of PMN ultimately responding to infection was equal to, or greater than, that in CBA/J mice, fewer macrophages accumulated. A/J mice failed to clear relatively low doses of the organisms and died. In vitro, chemotactic responses to both serum- and bacteria-derived attractants were similar for macrophages from A/J and CBA/J mice but were reduced for PMN from A/J mice. PMN and macrophages from the two mouse strains phagocytosed and killed spores in vitro to a similar extent, although killing by A/J PMN could be blocked by prior uptake of large numbers of killed spores. Thus susceptibility to lethal infection with Sterne strain correlated with the delayed influx (PMN) and reduced accumulation (macrophages) of phagocytes at the initial site of infection, but not with defective in vitro uptake or killing of spores.

Welkos SL, Vietri NJ, Gibbs PH. (1993). Non-toxigenic derivatives of the Ames strain of Bacillus anthracis are fully virulent for mice: role of plasmid pX02 and chromosome in strain-dependent virulence. Microb Pathog 14(5): 381-388	Division of Bacteriology, United States Army Medical Research Institute of Infectious Diseases, Frederick, MD 21702-5011. The toxin-encoding plasmid pX01 and capsule-associated plasmid pX02 are required for full virulence of Bacillus anthracis in some animals. However, the non-toxigenic pX01-cured derivatives of certain anthrax strains are not completely attenuated for mice, and their virulence is strain-dependent. The strain-related differences were partially associated with plasmid pX02 as demonstrated by pX02 transductants of the attenuated vaccine strain UM23-1 cured of pX01. To determine the virulence of non-toxigenic variants of virulent strains, we isolated pX01- derivatives of the Vollum 1B strain and the more 'vaccine-resistant' Ames strain which carried pX02 from either Ames or Vollum 1B. The 50% lethal dose (LD50) values of the derivatives of both strains which carried the Ames pX02 were not significantly different from the LD50s of the pX01+ pX02+ strains (and were lower than those of pX01+ pX02- strains). pX02+ derivatives of strain UM23-1 were less virulent than the comparable Ames and Vollum 1B strain derivatives, emphasizing a role for chromosomal loci in the virulence of the latter two strains. Non-toxigenic isolates which carried the Ames pX02 were more virulent for CBA/J mice than those with Vollum 1B pX02, and the differences were mouse strain-dependent. The pX01- pX02+ strains multiplied and achieved high concentrations systemically.
Westwood A, Healey GD, Williamson ED, Eyles JE. (2005). Activation of dendritic cells by microparticles containing *Bacillus anthracis* protective antigen. Vaccine.. 23: 29, 3857-3863.	We have carried out an in vitro investigation into the mechanism by which microencapsulation enhances the immunogenicity of recombinant protective antigen (rPA) from *Bacillus anthracis*. Murine bone marrow derived dendritic cells (DC) were cocultured with soluble and microencapsulated rPA and the activation status of the cells monitored using FACS. As compared with soluble rPA, it was found that coculture of DC with rPA-loaded microparticles stimulated higher levels of MHC II, CD54, CD80 and CD86 expression ($p<0.05$). To investigate the longevity of antigen presentation, splenocytes from naive mice were pulsed overnight with 3H-thymidine following 1, 3 or 6 days coculture with DC transiently exposed to soluble or microencapsulated rPA. Splenocyte proliferation was more pronounced, and continued for a more protracted period, if the 'feeder' cells were exposed to microencapsulated antigen as compared with soluble antigen or 'empty' microspheres. To this end, our findings indicate that microsphere uptake increases the surface expression of MHC and co-stimulatory molecules on DC and can facilitate prolonged presentation of antigen to T-cells, possibly by acting as an intracellular depot.
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White R (1998). With no recognition of the event, how do you plan a community development program? Aust J Emergency Management Winter: 28-31	Events associated with outbreak of anthrax in dairy cattle in Tatura in 1997.
Whitford HW (1984). Anthrax. *In*: Handbook of Zoonoses, edited by JS Steele, Section A: Bacterial, Rickettsial, and Mycotic Diseases, CRC Press, Boca Raton, Florida, pages 31–66.	
Whitford HW (1990). Incidence of anthrax in the USA: 1945 -- 1988. Salisbury Medical	Anthrax decline in US attributed to (1) use of Sterne vaccine, (2) use of antibiotics, especially penicillin, (3) prompt

Bulletin, Supplement 68: 5-7	diagnosis and regulatory activities, (4) reduction in areas used for grazing livestock.
Whitford HW (1994). Anthrax. In: Handbook of Zoonoses, 2nd edition (GW Beran and JS Steele, eds), Section A: Bacterial, Chlamydial and Mycotic, CRC Press, Boca Raton, 61–82.	
Whitford HW. (1978). Factors affecting the laboratory diagnosis of anthrax. Journal of the American Veterinary Medical Association.. 173: 11, 1467-1469.	Samples must be obtained quickly from the carcass, especially in hot weather. Experiments have shown that the vegetative organisms die at ambient temperatures of 25-30 deg C, and few could be isolated from carcasses left for 80 hours. At temperatures of 5-10 deg C the organism could be recovered four weeks after death. Contamination with other organisms is rapid, especially in fluids from the nose and perineal area. The jugular vein is the recommended site for obtaining blood samples. Various stains used for identifying B. anthracis are described.
Whitford HW. (1984). Anthrax: update and overview. Proceedings of Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians.. 26: 1-11.	A survey of diagnostic laboratories and/or state veterinarians was conducted to determine the incidence of laboratory-confirmed cases of anthrax in livestock in the U.S.A. Only 7 states had laboratory-confirmed cases of anthrax during the period 1980-1983: California 1 case in 1980, Colorado small outbreak in 1980, and New Mexico 1 case in 1980. Idaho 1 case in 1982; South Dakota 7 cases in 1980, 5 in 1981, 1 in 1982; North Dakota approximately 2 cases per year since 1980, except none in 1983; Texas 4 cases in 1980, 2 in 1981, 1 in 1982, 6 in 1983.
Whiting GC, Rijpkema S, Adams T, Corbel MJ. (2004). Characterisation of adsorbed anthrax vaccine by two-dimensional gel electrophoresis. Vaccine.22(31-32): 4245-4251	Division of Bacteriology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK. gwhiting@nibsc.ac.uk The current UK anthrax vaccine is an alum precipitate prepared from static culture filtrate of the avirulent, unencapsulated Sterne strain of *Bacillus anthracis*. Protective antigen (PA) is regarded as the major immunogen in the vaccine and production conditions are intended to maximize the PA content. However, the precise composition of the vaccine is unknown and there are concerns that the observed side effects of vaccination may be caused by residual enzymatically active toxin components. Two-dimensional gel electrophoresis (2DGE) was used to define the protein components of the current UK anthrax vaccine. Consistency of composition was assessed by examining batches spanning 14 years of vaccine production. The reproducibility of the 2DGE technique was assessed by repeated analysis of selected vaccine batches. For two recently produced batches, between 86.7 and 88.8% of the spots could be matched. However, for one older batch, reproducibility of the spot pattern was considerably less, with a mean similarity of 53.4%. This difference may be explained by a change in production or because of decay during storage. Variation between the recently produced batches ranged from 72.9 to 84.3%, whereas the similarity between these and old batches was comparatively low at between 30 and 59%. Our results demonstrate that, as expected, the major antigen present in the vaccine is PA. The 83 and 63 kDa species are dominant but there are numerous lower molecular weight fragments resulting from proteolytic cleavage. In addition, we have established the presence of the toxin components, oedema factor and lethal factor, and S-layer proteins, EA1 and SAP. Mass spectrometry has also enabled us to identify several bacterial cell-derived proteins present in the vaccine, including PA, enolase, fructose-bisphosphate aldolase, nucleoside diphosphate kinase and a 60 kDa heat shock protein. The use of proteomics can provide useful information on the antigenic make up of this vaccine and the consistency of vaccine production.
Whitney EAS, Beatty ME, Taylor TH, Weyant R, Sobel J, Arduino MJ, Ashford DA (2003). Inactivation of *Bacillus*	Review of heat, chemical and gaseous sterilization, radiation (UV, gamma)

anthracis spores. Emerg Infect Dis 9: 623-627	
WHO (1957). Joint FAO/WHO expert committee on milk hygiene. First report. WHO Technical report series No. 124.	Pp 14-15 refer to anthrax. 'Fortunately, cattle infected with anthrax do not usually excrete the bacillus in their milk since milk secretion stops abruptly.'
WHOCC (2005). World anthrax data site. World Health Organisation Collaborating Center for Remote Sensing and Geographic Information Systems for Public Health. http://www.vetmed.lsu.edu/whocc/mp_world.htm	
Wigelsworth DJ, Krantz BA, Christensen KA, Lacy DB, Juris SJ, Collier RJ (2004). Binding stoichiometry and kinetics of the interaction of a human anthrax toxin receptor, CMG2, with protective antigen. J Biol Chem 279: 23349–56.	[Scobie et al 2005, 10]
Wild MA, Xin H, Maruyama T, et al. (2003). Human antibodies from immunized donors are protective against anthrax toxin in vivo. Nat Biotechnol 21: 1305–6.	[Scobie et al 2005, 9]
Williams BM, Andrews AH. (1992). Bacterial conditions. Bovine medicine: diseases and husbandry.. Blackwell Scientific Publications Ltd, Oxford, UK:. 551-573.	
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Williams DR, Rees GB, Rogers ME. (1992). Observations on an outbreak of anthrax in pigs in north Wales. Veterinary Record.. 131: 16, 363-366	
Williams NF (1932). Anthrax. JAVMA 81: 9-25	
Williams RC, Rees ML, Jacobs MF, Pragai Z, Thwaite JE, Baillie LW, Emmerson PT, Harwood CR. (2003). Production of *Bacillus anthracis* protective antigen is dependent on the extracellular chaperone, PrsA. J Biol Chem. 278(20):	School of Cell and Molecular Biosciences, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4HH, United Kingdom. Protective antigen (PA) is a component of the *Bacillus anthracis* lethal and edema toxins and the basis of the current anthrax vaccine. In its heptameric form, PA targets host cells and internalizes the enzymatically active components of the toxins, namely lethal and edema factors. PA and other toxin components are secreted from B. anthracis using the Sec-dependent secretion pathway. This requires them to be translocated across the cytoplasmic membrane in an

18056-18062	unfolded state and then to be folded into their native configurations on the trans side of the membrane, prior to their release from the environment of the cell wall. In this study we show that recombinant PA (rPA) requires the extracellular chaperone PrsA for efficient folding when produced in the heterologous host, B. subtilis; increasing the concentration of PrsA leads to an increase in rPA production. To determine the likelihood of PrsA being required for PA production in its native host, we have analyzed the B. anthracis genome sequence for the presence of genes encoding homologues of B. subtilis PrsA. We identified three putative B. anthracis PrsA proteins (PrsAA, PrsAB, and PrsAC) that are able to complement the activity of B. subtilis PrsA with respect to cell viability and rPA secretion, as well as that of AmyQ, a protein previously shown to be PrsA-dependent.
Williams RP (1986). *Bacillus anthracis* and other aerobic spore-forming bacilli. In Infectious diseases and medicial microbiology, edited by AI Braude, CE Davis, J Fierer, 2nd edition, WB Saunders Co, Philadelphia, pp 270-278	
Williamson ED, Beedham RJ, Bennett AM, Perkins SD, Miller J, Baillie LWJ. (1999). Presentation of protective antigen to the mouse immune system: immune sequelae. J Appl Microbiol 87: 315-317	
Williamson ED, Bennett AM, Perkins SD, Beedham RJ, Miller J, Baillie LWJ. (2002). Co-immunisation with a plasmid DNA cocktail primes mice against anthrax and plague. Vaccine.. 20: 23/24, 2933-2941.	The protective antigen (PA) of *Bacillus anthracis* and the V antigen of Yersinia pestis are potent immunogens and candidate vaccine subunits. When plasmid DNA encoding either PA or V antigen was used to immunize the Balb/c mouse, a low serum IgG titre was detected (log10 1.0 or less) which was slightly increased by boosting with plasmid DNA. However, when mice immunized with plasmid DNA were later boosted with the respective recombinant protein, a significant increase in titre (up to 100-fold) was observed. Mice primed with a combination of each plasmid and boosted with a combination of the recombinant proteins, were fully protected (6/6) against challenge with Y. pestis. This compared favourably with mice primed only with plasmid DNA encoding the V antigen and boosted with rV, which were partially protected (3/6) against homologous challenge or with mice primed and boosted with plasmid DNA encoding the V antigen which were poorly protected (1/6). Combined immunization with the two plasmid DNA constructs followed by boosting with a combination of the encoded recombinant proteins enhanced the protective immune response to Y. pestis compared with priming only with plasmid DNA encoding the V antigen and boosting with rV. This enhancement may be due to the effect of CpG motifs known to be present in the plasmid DNA construct encoding PA.
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Wojnarowicz C, Ngeleka M, Sawtell SS, McLane JR. (2004). Saskatchewan: unusual winter outbreak of anthrax. Canadian Veterinary Journal.. 45: 6, 516-517.	This article describes an outbreak of anthrax in a 170-head commercial beef herd in Saskatchewan, Canada, during the winter in January 2002. Affected animals were dyspnoeic and moribund. Postmortem examination of cows that had suddenly died revealed blood-tinged fluid in the nostrils and anus; petechial and ecchymotic haemorrhages on the surfaces of the heart, lungs, spleen and intercostal muscles. Histopathological analyses conducted on these samples yielded high numbers of bacteria, which were identified as *Bacillus anthracis*. The infection originated from contaminated feed and soil. The herd was quarantined and, together with some horses in the same farm, was vaccinated against anthrax. Contaminated carcasses, soil and feed were subsequently burned and buried. The people in the farm were subjected to postexposure treatment, while their dog, who became sick after ingesting a portion of an anthrax-positive cow, was treated with injectable penicillin and amoxicillin and recovered completely. This report emphasizes the need for constant vigilance for animal diseases.
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Young JA, Collier RJ. (2002). Attacking anthrax. Scientific American 286 (3) 35-45	[protective antigen etc]

Young JB (1975). Epizootic of anthrax in Falls County, Texas. JAVMA 167: 842-843	Deaths in bulls > cows 'one can assume that its use [Sterne vaccine] stopped th epizootic'
Yurusun M, Yumusak M. (1989). Use of the indirect microhaemagglutination method to measure antibodies after vaccination against anthrax. Etlik Veteriner Mikrobiyoloji Dergisi.. 6: 5, 59-72.	[Turkish]
Zahavy E, Fisher M, Bromberg A, Olshevsky U. (2003). Detection of frequency resonance energy transfer pair on double-labeled microsphere and *Bacillus anthracis* spores by flow cytometry. Applied and Environmental Microbiology. . 69: 4, 2330-2339.	Development of an ultrasensitive biosensor for biological hazards in the environment is a major need for pollutant control and for the detection of biological warfare. Fluorescence methods combined with immunodiagnostic methods are the most common. To minimize background noise, arising from the unspecific adsorption effect, we have adapted the FRET (frequency resonance energy transfer) effect to the immunofluorescence method. FRET will increase the selectivity of the diagnosis process by introducing a requirement for two different reporter molecules that have to label the antigen surface at a distance that will enable FRET. Utilizing the multiparameter capability of flow cytometry analysis to analyze the double-labeling/FRET immunostaining will lead to a highly selective and sensitive diagnostic method. This work examined the FRET interaction of fluorescence-labeled avidin molecules on biotin-coated microspheres as a model system. As target system, we have used labeled polyclonal antibodies on *Bacillus anthracis* spores. The antibodies used were purified immunoglobulin G (IgG) molecules raised in rabbits against B. anthracis exosoporium components. The antibodies were fluorescence labeled by a donor-acceptor chromophore pair, alexa488 as a donor and alexa594 as an acceptor. On labeling the spores with alexa488-IgG as a donor and alexa594-IgG as an acceptor, excitation at 488 nm results in quenching of the alexa-488 fluorescence (Eq=35%) and appearance of the alexa594 fluorescence (Es=22%), as detected by flow cytometry analysis. The FRET effect leads to a further isolated gate (FL1/FL3) for the target spores compared to competitive spores such as B. thuringiensis subsp. israelensis and B. subtilis. This new approach, combining FRET labeling and flow cytometry analysis, improved the selectivity of the B. anthracis spores by a factor of 10 with respect to B. thuringiensis subsp. israelensis and a factor of 100 with respect to B. subtilis as control spores.
Zegers ND, Kluter E, van Der Stap H, van Dura E, van Dalen P, Shaw M, Baillie L. (1999). Expression of the protective antigen of *Bacillus anthracis* by *Lactobacillus casei*: towards the development of an oral vaccine against anthrax. J Appl Microbiol 87(2): 309-314	TNO Prevention and Health, Division of Immunological and Infectious Diseases, Leiden, The Netherlands. nd.zegers@pg.tno.nl *Bacillus anthracis* is the causative organism of the disease anthrax. The ability of the organism to form resistant spores and infect via the aerosol route has led to it being considered as a potential biological warfare agent. The current available human vaccines are far from ideal, they are expensive to produce, require repeated doses and may invoke transient side-effects in some individuals. There is also evidence to suggest that they may not give full protection against all strains of B. anthracis. A new generation of anthrax vaccine is therefore needed. The use of Lactobacillus as a vector for expression of heterologous proteins from pathogens supplies us with a safe system, which can be given orally. Lactobacilli are commensals of the gut, generally regarded as safe and have intrinsic adjuvanticity. Oral vaccines may stimulate the mucosol immune system to produce local IgA responses in addition to systemic responses. These vectors are delivered at the mucosal surface, the site where the infection actually occurs and where the first line of defence lies. The gene encoding the protective antigen (PA) of B. anthracis, an immunogenic non-toxic component of the two toxins produced, is being cloned into different homologous vectors and subsequently transformed to various Lactobacillus strains. High intracellular expression levels for the PA in Lact. casei were achieved. Mucosal antigen

	presentation and humoral and cellular immune responses following immunization with transformants expressing PA in various ways (intracellular, surface-anchored and extracellular) are being studied.
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Zhang S, Finkelstein A, Collier RJ. (2004). Evidence that translocation of anthrax toxin's lethal factor is initiated by entry of its N terminus into the protective antigen channel PNAS 101: 16756-16761	
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Zhao Qing; Xiao LuZhong. (2004). Establishment of a detection method of *Bacillus anthracis* in raw wool, skin and soil by PCR. Chinese Journal of Veterinary Science and Technology. 34: 2, 7-11.	A PCR technique for the detection of *Bacillus anthracis* gene Ba813 was established and tested using artificially contaminated raw wool, skin and soil. Results showed that the PCR can detect sensitively and specifically 452 spores of Sterne strain in 0.5 g raw wool, skin and soil samples, 688 spores of Pasteur II strain in 0.5 g raw wool, 1032 spores of Pasteur II strain in 0.5 g skin and 1376 spores of Pasteur II strain in 0.5 g soil. The PCR can be used for the detection of B. anthracis in wool, skin and soil.

Appendix 5

FAO ANIMAL PRODUCTION AND HEALTH PAPER 87

MANUAL FOR THE PRODUCTION OF ANTHRAX VACCINE

Dr R.P. Misra

M-27
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PREFACE

This manual provides a comprehensive description of standard methods for the production of vaccines against anthrax. All steps of the procedure are taken up, from the choice of a vaccine strain to the shipping of the final product, with all relevant technical details. Anthrax is a multispecies disease caused by spore-forming bacteria. Spores enable organisms to survive for long periods in the natural environment and particularly in soil which is a natural reservoir for both diseases.

The original vaccine strain can release its immunogenic properties upon repeated subcultures so that it is necessary to use a "seed system" strategy, whereby the master seed-lot is controlled for efficacy before further processing. The vaccine is evaluated for both potency and innocuity in laboratory animal models, according to standardized protocols.

We hope that this manual will assist vaccine production units of developing countries in manufacturing anthrax vaccines of high quality standards.

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General laboratory procedures

ENVIRONMENTAL CONTROL

Anthrax spore vaccine is a living vaccine. To avoid contamination during its preparation, it is necessary to keep the bacterial population in the environment of the work area to a minimum. This can be best achieved by providing a filtered air supply and laminar flow cabinets. In the absence of filtered air supply, regular fumigation of laboratories and work cabinets should be carried out to keep airborne microbes to a minimum.

All the work surfaces of the laboratories should be kept clean and dust free at all times, the benches and work area should be swabbed regularly with a disinfectant such as methylated spirit or chloros at the beginning and at the end of each working day.

Formaline is commonly used for fumigation. It works best at temperatures above 24°C and at humidity above 65 percent. Fumigation should be carried out at the end of the working day. For one cubic metre space, a mixture of 13 ml of formaline (37 percent) and 6.5 g of potassium permanganate is placed in a Petri dish and the area should be sealed. Following overnight fumigation, seals are removed and the room can be used for work after removing the excess of formaline vapour by an exhaust fan.

Ultraviolet irradiation is often employed for the disinfection of the cabinets. The ultraviolet lamp should be kept clean and its bacteriocidal activity regularly checked, since its germicidal power deteriorates after some time, although the discharge of the rays continues. The cabinets and work stations should be regularly checked for their efficiency.

The level of airborne microbes should be regularly monitored in laboratories, cabinets and work stations. This is carried out by opening blood agar Petri dishes for a specified time in a specified position. After appropriate incubation of the plates, colony counts are made.

Adequate staff should be provided to avoid the necessity for the same staff to work in the production of other vaccines on the same working day. Staff must wear sterile protective clothing, gowns, head covers and face masks, and must change their shoes before entering the laboratory. Protective clothing should be changed daily, and should be autoclaved after use or discarded if disposable.

Visitors and persons not directly concerned with production should not be allowed to enter the vaccine production laboratories. Service personnel having occasional duties inside the laboratories should wear protective clothing before entering.

Pathological specimens for diagnosis should be processed only in separate areas not used for manufacturing biological substances.

PRECAUTIONS AND LABORATORY DISCIPLINE

Anthrax organisms are among the dangerous group of bacteria. There is a risk of contracting the infection particularly while handling virulent strains and artificially infected animals.

However simple precautions will protect laboratory workers from infection with anthrax. Laboratory discipline is extremely important and it is recommended that:

- anthrax cultures should only be handled by personnel trained in microbiological techniques;
- protective clothing and face masks should be used while handling the cultures and infected materials. These should be sterilized after completion of the work;
- all cultures and infected materials should be handled in safety cabinets;
- after completion of the work, infected materials, culture plates, tubes, pipettes and other contaminated materials should either be discarded into leakproof disposable containers and incinerated, or decontaminated by autoclaving;
- reusable glassware should be soaked in disinfectant or autoclaved before cleaning;
- eating, drinking, or smoking should not be allowed in the laboratory;
- pipetting by mouth should be prohibited.

Experimental animals

For vaccine production and quality control, it is essential that arrangements should be made for the regular supply of good quality experimental animals from a known source. Specific pathogen free animals are ideal for the production and testing of vaccines. However, in their absence the animals reared in isolation obtained from disease free stocks can be used.

Laboratory animals or other animals used for production and quality control of vaccines should be provided with adequate housing. The breeding colony for healthy laboratory animals and experimental animals should be located in separate buildings away from the vaccine production unit. The animal house should be designed and constructed in such a way that it can be easily cleaned, and it should be free from insects. It should be provided with facilities for feed storage, washing and disinfection of cages, an isolation unit for quarantine, a post-mortem room and an incinerator for disposal of waste and dead animals.

In ideal conditions, experimental animal rooms should be provided with filtered air exhaust systems with absolute filters to prevent escape of virulent organisms, as well as facilities for effective sterilization of effluent water, leftover feed, fodder, urine and faeces by disinfection and incineration. In the absence of the above facilities, the experimental animal house should be provided with birdproof netting to prevent spreading of virulent organisms by free flying birds, and a drainage system where effluents should be properly disinfected. Animal sheds and equipment should be disinfected at regular intervals.

The efficacy of the vaccine depends upon the results of animal experiments.
Thus:

- the animals must be kept under conditions in which they will remain healthy and will not be subjected to cross infection, and should not be overcrowded;
- they must be provided with good hygienic conditions with sufficient light and ventilation;
- they must be given a good balanced diet and abundant water supply;
- animal sheds and cages must be cleaned daily and disinfected periodically.

For the production and testing of anthrax spore vaccine, guinea pigs, sheep and goats are needed.

GUINEA PIGS

Guinea pigs used in vaccine production and testing should be obtained from a healthy colony. Animals weighing approximately 400 to 500 g are suitable for quality control tests. Guinea pigs utilized in testing of vaccine should be kept for a minimum period of three days to adapt to the new environment of shed where the tests are to be carried out. The temperature of guinea pigs should be recorded twice a day, morning and evening. Any guinea pig showing a temperature of above 40°C should not be used and replaced.

SHEEP

One to two year-old healthy unvaccinated sheep weighing approximately 20 kg are suitable for the quality control tests. Sheep should be kept for one week to adapt to the new environment of the sheds where the tests are to be carried out. Their daily morning and evening temperature should be recorded. Any sheep showing an abnormal temperature should not be used and should be replaced.

GOATS

One-year-old healthy unvaccinated goats, weighing approximately 20 kg, are suitable. They should be kept for one week to adapt to the new environment of sheds where the tests are to be carried out. The temperature of these goats should be recorded twice a day. Any goat showing a temperature above 40°C should not be used and should be replaced.

Media

For the production of anthrax spore vaccine solid medium is used. Two types of solid media, i.e. nutrient agar and casein digest agar are used for the vaccine production. Yield of viable spores in nutrient agar medium is satisfactory but comparatively lower than casein digest agar medium. Vaccine produced from casein digest agar medium is safe and yield of viable spores is also higher, and this medium has been recommended by WHO in requirements for production of anthrax spore vaccine, live (veterinary), 1967.

NUTRIENT AGAR

This is the simplest medium used for production of anthrax spore vaccine. The method of its preparation is given in Appendix 1.

CASEIN DIGEST AGAR

The main constituent of this medium is a tryptic digest of casein. The medium is buffered and salts are added to promote sporulation. The formula and method of its preparation are given in Appendix 1.

MEDIA FOR TESTING BACTERIAL CONTAMINATION

For testing absence of contamination, fluid thioglycolate, soybean-casein digest and motility media are recommended. The formula and methods of their preparation are given in Appendixes 1 and 2.

Strains of Bacillus anthracis

STRAIN FOR VACCINE PRODUCTION

In the past capsule forming Pasteur strains were used for the production of anthrax vaccines. These vaccines induced severe reactions and were not safe in all animals. During the last 40 years, capsule forming strains have been replaced by uncapsulated attenuated strains derived from a virulent strain. In most countries, anthrax spore vaccine is prepared from an uncapsulated avirulent strain 34 F 2 of *B. anthracis*. This strain was originally isolated by Sterne (1937) from a virulent strain. Its isolation and history are briefly described below:

The virulent strain of *B. anthracis* grown on nutrient agar at 37°C in normal atmospheric conditions produces frosted glass-type rough colonies of medusa head type, while the virulent strain grown on 50 percent horse serum agar and incubated at 37°C in an atmosphere of 30 to 50 percent carbon dioxide produces smooth raised mucoid colonies with clearly defined edges. These colonies are quite different from those grown in ordinary conditions on nutrient agar. The organisms from mucoid colonies are capsulated and are in short chains. After further incubation for 3 to 4 days, examination under a low power objective shows that some of these mucoid colonies have a fan-shaped outgrowth at their outer edges. These outgrowths have a filamentous appearance and bacilli are in long chains and do not show capsulation. These variants on subculture on ordinary or enriched nutrient agar produce typical anthrax colonies of frosted glass appearance and hairlike structure, irrespective of whether they are grown in an atmosphere containing 30 to 50 percent carbon dioxide or not. On repeated subcultures or passage through the animals, organisms do not change their characteristics and show no tendency to revert to the capsulated virulent form. In moderate doses these variants are not virulent to mice and guinea pigs. However, massive doses can kill guinea pigs and mice, but even then no trace of capsulation is observed.

The main advantages of the 34 F 2 strain are that it is stable, that it cannot produce capsule *in vitro*, that it is safe in all animals, that it induces solid immunity lasting for one year, that immunogenicity can be tested in laboratory animals.

This strain has been found to be safe and effective in protecting animals and is used in most countries of the world for the production of anthrax spore vaccine. The preservation of the strain is described later.

STRAINS FOR TESTING POTENCY

For testing the potency of anthrax spore vaccine, highly virulent challenge strains are required. Two types of challenge strains, i.e. guinea pig challenge strain 17 JB and a virulent strain are used. In most countries the potency of anthrax spore vaccine is carried in guinea pigs but in a few countries sheep and goats are used. It is recommended to carry out the potency test of anthrax spore vaccine in guinea pigs.

Guinea pig challenge strain (17 JB)

Guinea pig challenge strain was derived by Sterne by continuous passage in guinea pigs of the Pasteur II strain until virulence could not be further enhanced. This strain is consistently virulent for guinea pigs but not for rabbits, domestic animals or man. It is used as a challenge strain for testing the potency of anthrax spore vaccine in guinea pigs. This strain can be obtained from the Central Veterinary Laboratory, Weybridge, Surrey, UK.

It is recommended to preserve the 17 JB strain by freeze-drying. For the maintenance of maximum virulence, passage the strain at least once in guinea pigs before freeze-drying. Propagate the organisms on nutrient agar and inoculate the guinea pigs subcutaneously. Isolate the organisms from heart blood or spleen just before death.

Propagate the strain on nutrient agar. Harvest in physiological saline solution when there is maximum sporulation. Test the purity by smear examination and motility test. The detailed method of propagation and purity test is given later.

Mix the spore suspension with equal amounts of stabilizers (Appendix 3) and freeze-dry in 1 ml quantities in ampoules or vials. Carry out the primary drying for 18 hours and the secondary for four hours. Seal the ampoules or vials under vacuum. Test the viability of spores on media. Label the containers and store at -20°C in the quality control laboratory. Freeze-dried spores stored at -20°C retain viability for years.

Determine the minimum lethal dose (MLD) of the freeze-dried spores in guinea pigs weighing 400 to 500g. Prepare tenfold dilutions in physiological saline solution. Inoculate 1.0 ml subcutaneously in guinea pigs and observe for ten days. Record the death of guinea pigs due to anthrax and determine the MLD.

For testing the potency of anthrax spore vaccine, it is recommended to use at least 100 MLD of guinea pig challenge 17 JB strain as per recommendations of the British Pharmacopoeia (Veterinary), 1985.

Virulent strain

In certain countries, potency testing of anthrax spore vaccine is carried out in sheep. For this purpose a highly virulent strain of *B. anthracis* is required. For the maintenance of maximum virulence, the strain should be given three quick passages in sheep by using blood. Blood from the third passage should be collected just before death and cultured on nutrient agar, spores freeze-dried and stored at -20°C or lower in the quality control laboratory. Determine the MLD of the freeze-dried virulent spores in sheep. Prepare tenfold dilutions in physiological saline solution. Inoculate 1 ml subcutaneously in sheep and observe for ten days. Record the death of sheep due to anthrax and calculate the MLD. One hundred MLD of virulent strain should be used as a challenge dose in sheep for estimating the potency of anthrax spore vaccine.

Preparation and control of vaccine seed-lots

The preparation of good seed is a prerequisite for the production of a safe and effective vaccine. The strain used for the production of vaccine seed-lots must be a standard strain capable of yielding safe and immunogenic vaccine that meets all the manufacturing requirements as laid down by WHO for the anthrax spore vaccine, live (veterinary) 1967. It is therefore essential that the seed-lot be subjected to a full range of tests, i.e. identity, freedom from contamination, safety, potency and stability, prior to use in vaccine production. Strain 34 F 2 (Sterne) is a suitable strain and is being used in most countries. This strain can be procured from the Central Veterinary Laboratory, Weybridge Surrey, UK.

The attenuated strains loose their antigenicity gradually on repeated subcultures on artificial media. It is therefore essential that for vaccine production, culture should be kept within three passages from the original seed obtained from the reference laboratory. For this reason a large stock of freeze-dried seed should be prepared and stored at -20°C or lower. This can

be used for several years. It is therefore necessary that the production of anthrax spore vaccine be based on seed-lot systems. Two types of seed-lots, i.e. master seed and working seed, should be prepared for the production of anthrax spore vaccine. The working seed-lot is prepared from master seed. This seed-lot system ensures that over a period of several years, the seed strains themselves are not subcultured. It is strongly recommended that the seed-lot should be prepared in the production laboratory and then submitted to the quality control laboratory for testing. Full records should be kept of the origin, passage and storage of seed-lots including the date of subculture and medium used for its propagation. The use of seed-strain for production lots should also be recorded. A new batch of seed-lot should be prepared before its complete exhaustion, because if the passage material is later found unsuitable, it will be necessary to revert to a previous passage level. Seed strain should be maintained both in freeze-dried form and on artificial media. In the event of damage of one, an alternative supply of culture will be available. Seed-lots should be properly labelled and stored in the production laboratory at 4°C in a freezer or refrigerator that does not contain contaminating materials.

PREPARATION OF MASTER SEED-LOT

The ampoule of vaccine seed obtained from the reference laboratory should be opened as follows:

- Clean and sterilize the ampoule with cotton soaked in rectified spirit and allow it to evaporate.
- Tap the ampoule gently on the table top to collect the freeze-dried material at the bottom.
- Saw the ampoule with an ampoule file 1 cm from the top.
- Heat a glass rod, touch one end of the file mark on the ampoule.
- When a crack appears, hold the ampoule horizontally, gently remove the top end, taking precautions that no glass particles fall into the material and no material falls out.

Reconstitute the freeze-dried material in 1 ml sterile physiological saline solution, plate it on nutrient agar Petri dishes and incubate at 37°C for 24 hours. Fish out the typical anthrax colonies, plant on nutrient agar slants and incubate at 37°C for 24 hours. Check the purity of the slants by smear examination after staining with Gram's method and motility test. The procedure to carry out the motility test is described in Appendix 2.

Suspend the growth of the pure slants in 4 ml sterile physiological saline solution. Inoculate 2 ml in each nutrient agar Roux flask. Incubate the Roux flasks at 37°C for 72 hours and then for four days at room temperature, wash the growth of each flask with 10 ml physiological saline solution with the help of glass beads. Harvest the growth from each Roux flask separately. Check the purity of spore suspension by smear examination after staining with Gram's method and motility test.

Pool the pure spore suspensions in an Erlenmeyer flask, add an equal volume of sterilized stabilizer and freeze-dry. The preparation of the stabilizer is given in Appendix 3. Check the vacuum of the freeze-dried containers and discard those that do not have a vacuum. Check the purity of the freeze-dried spore by smear examination after staining with Gram's method and motility test. Lable each container and store at -20°C or lower. This is designated as the master seed-lot which can be used for several years.

PREPARATION OF WORKING SEED-LOT

The working seed-lot is prepared from the master seed-lot. Check the vacuum of the container of the freeze-dried master seed-lot and reconstitute in 1 ml sterile physiological saline solution, and inoculate several nutrient agar slants prepared in screw-capped tubes. Incubate at 37°C for 24 hours. Check the purity by smear examination and motility test. Store pure slants at +4°C. These culture slants are used for the preparation of inoculum for the production of vaccine up to six months.

It should be noted that the working seed-lot should not be more than three passages removed from the well characterized master seed-lot, and that vaccines should be made from the working seed-lot without additional passage.

CONTROL OF SEED-LOT

Control of the seed-lot is carried out once in the beginning when seed-lots are prepared.

Tests for bacterial contamination

Test the purity of the seed-lot by morphological, cultural and motility tests.

Morphological test. Prepare a smear from five samples of the seed-lot cultured in nutrient broth and nutrient agar, stain by Gram's method and examine under the microscope. The seed-lot must contain only *B. anthracis* and must be free from contaminants.

Cultural tests. Streak five nutrient agar plates with five samples of seed-lot and incubate at 24 to 48 hours at 37°C. Examine the morphology of colonies by naked eye and through a magnifying glass. Prepare a smear and examine under the microscope after staining with Gram's stain. The seed-lot must not contain any bacteria except *B. anthracis*.

Inoculate five samples of seed-lot into five Erlenmeyer flasks containing 50 ml of nutrient broth, incubate at 37°C and observe up to seven days. Test the purity by smear examination from each flask daily up to seven days after staining with Gram's stain. The seed-lot must contain only *B. anthracis*. Inoculate five samples of seed-lot into five Erlenmeyer flasks containing 50 ml of thioglycolate fluid medium and incubate at 37°C and observe daily up to seven days. Test the purity from each flask by smear examination daily up to seven days after staining with Gram's method. There must not be any growth in the thioglycolate broth as *B. anthracis* is an aerobe and does not grow in fluid thioglycolate medium.

Motility test. Test the motility of five samples of seed-lot by inoculating in nutrient broth and fluid thioglycolate medium. The seed-lot must be free from motile organisms.

Safety test

The seed-lot must be tested for its safety by preparing a batch of vaccine from it. The safety test should be carried out on sheep as per WHO requirements for anthrax spore vaccine, live (veterinary), 1967.

Inoculate three healthy, one- to two-year-old sheep which were not immunized against anthrax, with 5 000 million viable spores subcutaneously and observe for ten days. Record the temperature daily both in the morning and evening. The seed-lot is considered safe when none of the sheep show any severe reactions except for a 2–3°C rise in body temperature

which persists four to five days. At the site of inoculation there is an oedematous swelling which subsides within six to seven days.

Immunogenicity test

The seed-lot must be tested for its immunogenicity by preparing a batch of vaccine from this as per WHO requirements for anthrax spore vaccine live (veterinary), 1967.

As per recommendation of the British Pharmacopoeia (Veterinary), 1985, the kind of animals employed for testing the potency of anthrax vaccine depend on the strain used for production of vaccines. When the strain used for vaccine production is not lethal to guinea pig or mouse, carry out the potency test on guinea pigs. When the strain is lethal to the guinea pig but not to the rabbit, carry out the test in rabbits. When the strain is lethal to the rabbit, carry out the test in sheep. Strain 34 F 2 (Sterne) being not lethal to guinea pigs, it is recommended to carry out the test in guinea pigs. In some countries the potency test is carried out in sheep. Method of potency tests in guinea pigs and sheep are described.

Guinea pigs. Inoculate ten healthy guinea pigs weighing approximately 500 g with 5 million viable spores subcutaneously and observe for 21 days, and record their body temperature daily. None of the animals should show untoward reactions except for a 1–1.5°C rise in temperature. If more than two animals die from non-specific causes, retest the vaccine. Challenge all the immunized guinea pigs along with three controls with 100 MLD and three controls with 10 MLD of 17 JB virulent guinea pig strain of *B. anthracis*. Observe these animals for 10 days and record their body temperature. All the vaccinated guinea pigs should survive and show no untoward reactions except slight rise in their body temperature, while all the controls must die from anthrax. Repeat the test if one of the vaccinated animals dies. The seed-lot passes the test if there is a 100 percent protection of the vaccinated guinea pigs and 100 percent death of the controls from anthrax.

Sheep. Inoculate ten healthy two-year-old sheep weighing approximately 20 kg with 5 million viable spores subcutaneously and observe for 21 days and record their temperature daily. None of the animals should show any untoward reaction except for a 1–3°C rise in their temperature. If more than two animals die from non-specific causes, retest the vaccine. Challenge all the immunized sheep along with three controls with 100 MLD of virulent strain of *B. anthracis*. Observe the animals for ten days and record their temperature daily. The vaccinated sheep should not show any untoward reaction except for a slight rise in their temperature and should survive the challenge, while all the controls must die from anthrax. Repeat the test if one of the vaccinated sheep dies. The seed-lot passes the test if there is 100 percent protection of the vaccinated sheep and 100 percent death of the controls from anthrax.

Preparation and control of vaccine concentrate

PREPARATION OF VACCINE CONCENTRATE

Inoculation

Prepare casein digest agar medium in Roux flasks. From 50 standard Roux flasks approximately 100 000 doses of anthrax spore vaccine can be obtained. Incubate the Roux flasks containing the medium for 48 hours at 37°C before inoculation to ensure sterility. Place all the Roux flasks in the inoculation room, start the ultraviolet lamp and sterilize for 30

minutes. Remove the condensation water from each Roux flask under strict sterile conditions using a pipette. The medium should be dry before inoculation.

Prepare working seed from the master seed-lot before initiating production of anthrax spore vaccine. Place 10 ml of physiological saline solution in each tube of the working seed. Suspend the bacterial growth with a pipette. Inoculate 2 ml of bacterial suspension in each production flask very carefully by placing the mouth of the flask as near as possible to the burner with the assistance of a technician. Spread the inoculum evently on the surface of the agar by to and fro movements of flasks. Cover the mouth of each Roux flask with a paper cap. Incubate the flasks at 37°C for three days. Thereafter keep the flasks at room temperature for seven days. Check the smear of randomly selected flasks to find out the degree of sporulation. Harvest the growth from the Roux flasks when 90 percent of the organisms are in sporulated forms.

Harvesting

Pipette 20 ml of physiological saline solution and place a few glass beads in each Roux flask. Wash off the growth by to and fro movements of the flasks. Place the flasks on the working bench in an upright position. Harvest the spore suspension using a pipette under strict sterile conditions. Harvest the growth from ten Roux flasks in one Erlenmeyer flask of 250 ml capacity.

Purity test

Inoculate 0.1 ml of spore suspension from each Erlenmeyer flask in 100 ml of nutrient broth and incubate at 37°C overnight. Check the purity by staining the smear with Gram's staining and motility test. Discard the contaminated smears.

Glycerination

Determine the weight of a sterilized Erlenmeyer flask. Pool the pure spore suspension in the above flask and estimate the weight of the spore suspension. Add twice the weight of the bacterial suspension of sterile, pure and neutral glycerol. Add sufficient glass beads for mixing the glycerinated suspension. Shake the flask vigorously to ensure thorough mixing of the suspension. This suspension is referred to as “vaccine concentrate”.

Check the purity of vaccine concentrate as described earlier and keep it at 20°C for three weeks to destroy vegetative bacteria and weak spores, and store at 4°C. Alternatively to destroy the vegetative bacteria, heat the suspension at 65°C for one hour in a water bath and store the vaccine concentrate at 4°C for three weeks before carrying out the control tests.

CONTROL TESTS ON VACCINE CONCENTRATE

Tests for bacterial contamination

Carry out the tests for bacterial contamination of vaccine concentrate by morphological, cultural and motility tests as described before. The vaccine concentrate must not contain any bacteria other than *B. anthracis*.

Test for number of culturable spores

Determine the number of live spores in the vaccine concentrate by plating suitable dilutions on nutrient agar plates. The procedure is described in Appendix 4.

To avoid the possibilities of gross error, it is advisable to carry out the spore counts on at least three samples of the vaccine concentrate, and an average of three counts should be calculated. There is a tendency for the spores to clump; deposit them gradually in the vaccine concentrate and shake vigorously before taking samples for counting.

Safety test

Carry out the safety test on the species for which the vaccine is going to be used. If the vaccine is intended for several species, carry out the safety test on sheep or goats.

Inoculate two healthy sheep or goats subcutaneously with twice the dose recommended for vaccination, i.e. 20 million spores. Observe the animals for ten days and record their temperature both in the morning and evening. The vaccine concentrate passes the test if no abnormal systemic reaction is observed and none of the animals show severe reactions except a transient oedema at the site of inoculation which subsides in 3 to 5 days. Some animals show dullness and a 1–2°C rise in body temperature for three to five days, and become normal afterwards.

The development of progressive oedema at the site of inoculation indicates that the vaccine may be unsuitable for goats. The severity of the local reactions may vary according to the strain and adjuvant if any was used in the vaccine, but necrosis should not develop.

Potency test

Carry out the potency test in guinea pigs in parallel with the international reference vaccine procured from an international reference centre. The procedure is described in the section on control of the seed-lot. The vaccine passes the test if all the vaccinated animals survive and all controls succumb to the challenge from anthrax. Repeat the test if one of the vaccinated animals dies after the challenge.

DILUTION OF VACCINE CONCENTRATE

After determining the number of culturable spores in the vaccine concentrate, calculate the dilution factor depending upon the number of spores to be employed per dose.

It is recommended that the vaccine should contain not less than 10 million culturable spores per dose for cattle, buffaloes and horses and not less than 5 million for sheep, goats and pigs.

Dilute the vaccine concentrate in 50 percent glycerine saline solution pH 7.0. Prepare glycerine saline by mixing four parts neutral glycerine with six parts of physiological saline solution and sterilize in the autoclave at 121°C for 45 minutes.

ADJUVANT

In some laboratories 0.1 percent saponin is incorporated as an adjuvant in the vaccine. This is added during the dilution of the vaccine concentrate by glycerine saline.

Filling and containers

Transfer the required filling equipment, vials and stoppers after sterilization to the filling room which has been prepared before. Start the ultraviolet light and carry out the sterilization for 30 minutes. Filling operations should be conducted in such a way to avoid any contamination or alteration of the vaccine.

Transfer the vaccine concentrate into a sterile vaccine filling tank fitted with magnetic drive in the vaccine filling room. Add the required quantity of sterilized glycerine saline solution. Add saponin if required. Mix the vaccine slowly at room temperature for two hours.

Fill the vaccine in a multidose container in 10 or 20 ml vials using a pipetting machine under sterile conditions.

Close the vials with sterilized rubber stoppers and seal the vials with aluminium collars immediately, then place the vials in aluminium boxes and store at 4°C.

The quantity present in a single bulk container determines the batch size. If the final bulk is distributed in smaller containers, each of them is referred to as filling lot and is labelled individually. If the filling is carried out directly from the final bulk container, the number of vials filled in one session without stoppage is referred to as final lot or filling lot.

FREEZE-DRYING

In certain laboratories anthrax spore vaccine is prepared in freeze-dried form. For this purpose mix the spore suspension after harvesting with an equal volume of stabilizer. The composition of the stabilizer is given in Appendix 3. Distribute 1 ml in vials. Carry out the primary drying for 18 hours and secondary for four hours. Seal the freeze-dried containers under vacuum or under dry oxygen-free nitrogen. Test all the sealed containers for leaks, discard defective ones and store at 4°C or lower.

Control tests on final product

Apply the following control tests on each filling lot from the samples collected by random selection. If the vaccine is in freeze-dried form, apply hese tests on the vaccine reconstituted to the form in which it is to be used.

INSPECTION OF FINAL CONTAINERS

Inspect every container visually under proper illumination to detect the presence of foreign particles. Discard vials showing foreign particles, clumps and defects in closures.

IDENTITY TEST AND TESTS FOR ABSENCE OF BACTERIAL CONTAMINATION

Carry out the tests for identity and for absence of contamination on every final lot. According to the British Pharmacopoeia (1985), 1 percent of containers in a batch with a minimum of three or maximum of ten is considered suitable for carrying out the test. Carry out the tests by:

- morphological test, by examining the appearance of the bacilli in Gram's stained smears;
- cultural tests, by inoculating the vaccine on nutrient agar plates and nutrient broth;

- motility test.

The methods of the above tests are described in the section dealing with control of seed-lot, and motility test in Appendix 2. Vaccine should not contain any bacteria other than *B. anthracis*.

TEST FOR NUMBER OF CULTURABLE SPORES

Determine the number of culturable spores by plating suitable dilutions on nutrient agar. The procedure is described in Appendix 4.

The vaccine should not contain less than 10 million culturable spores per dose for cattle, buffaloes and horses and not less than 5 million per dose for sheep, goats and pigs.

In any case as per the British Pharmacopoeia (Veterinary) 1985, the number of live spores determined by a plate count method must not differ more than 20 percent from the standard numbers for a particular animal.

DETERMINATION OF pH

Determine the pH of each final lot of the vaccine. Standardize the pH meter with standard buffer solution of pH 5.0 and 7.0. Rinse the electrodes with distilled water, dry and test the pH of the vaccine. The pH value of anthrax spore vaccine should be 7.0±0.3.

INNOCUITY TEST

Carry out the innocuity test on each final lot of the vaccine. It is not necessary to carry out this test when the safety test is done on animals of the species for which the vaccine is intended to be used.

Inoculate two guinea pigs intraperitoneally with 0.2 ml of vaccine (reconstituted if freeze-dried) and two guinea pigs subcutaneously with 1 ml. Observe these animals for ten days. Vaccine passes the test if none of the animals shows signs of illness. If one of the animals dies or shows signs of ill health during observation, repeat the test. The vaccine lot passes the test if none of the animals in the second group dies or shows signs of ill health during observation.

RESIDUAL MOISTURE CONTENT FOR FREEZE-DRIED VACCINE

Determine the moisture content of each filling lot of freeze-dried anthrax spore vaccine. Dry the freeze-dried vaccine over phosphorous pentoxide under vacuum (0.01–0.03 mm Hg) at 56°C until a constant weight is obtained. Test the moisture content of five samples of freeze-dried vaccine. Moisture levels of freeze-dried anthrax spore vaccine should be less than 2 percent.

STABILITY TEST

Test the stability of every filling lot of freeze-dried anthrax spore vaccine by using the accelerated degradation test. Store five vials of vaccine at 37°C for four weeks. Determine the number of culturable spores at the end of the storage period in parallel with unstored vaccine and with the reference preparation. There should not be a drop in the number of

spores below the prescribed limits required for immunization on storage at 37°C for four weeks.

Carry out the long-term stability test by storing liquid vaccine at 4°C for 180 days or more. Determine the number of culturable spores before and after samples were held at 4°C in parallel with the reference preparation. The vaccine should contain the required number of culturable spores prescribed for immunization of animals.

RECORDS

RECORDS OF SEED-LOTS AND CULTURES

Maintain a complete passage history of the seed-lots and cultures used in production and quality control of anthrax spore vaccine. Label all cultures properly and store them at the appropriate temperature in an orderly manner. Keep a complete and accurate inventory of all stocks and of all the vials and ampoules issued or used.

PRODUCTION PROTOCOL AND DISTRIBUTION RECORDS

Keep the records of production and control protocols in such a way that it is possible to trace all steps in the manufacture and testing of a batch of vaccine. Keep the detailed records of sterilization of all apparatus and materials used in its manufacture. Keep the written records clearly indicating all steps in processing and filling including both the process controls and control test of the final product. Keep the written records of all the tests irrespective of their results throughout the dating period of each lot of vaccine and have them available at all times for inspection by the control authority.

Keep the distribution record in such a manner that it permits rapid recall of any particular batch.

A sample of a suitable summary protocol for production and control of anthrax spore vaccine is given in Appendix 5. The tests specified are based on the WHO requirements for anthrax spore vaccine, live (veterinary). The protocol is intended for the reporting of the data to the national control authority or to the authorities in other countries to whom the vaccine may be exported.

SAMPLES

At the time of release of a lot of anthrax spore vaccine sufficient quantities of samples from each final lot should be retained. Collect the samples by random selection, so that they are representative of the lot and store at 4°C. These samples are mainly required for the following essential purposes:

- To enable carrying out investigations in the event of complaints received from the field for a particular lot of anthrax spore vaccine and permit repetition of control tests if required.
- To ensure that anthrax spore vaccine has been stored correctly as a reference material and that the potency has been maintained throughout the storage period during the dating period of the vaccine.

Discard the retained samples when it is known that the complete lot has been distributed, used or destroyed at the end of the expiry period. To check the keeping quality particularly as regards potency of anthrax spore vaccine, it is beneficial to keep some samples from time to time beyond the expiry period.

LABELLING

Identify the anthrax spore vaccine, living, by label. The label on the container should show:

- the name of the vaccine - Anthrax spore vaccine, living;
- name and address of the manufacturer;
- lot number;
- dose;
- route of the administration: subcutaneous;
- storage temperature: +4°C;
- expiry date: six months from production.

The label on the packing or leaflet in the package should, in addition to the information shown on the label of the container, include the following details:

- Nature of amount of preservative or added substance present: in liquid anthrax spore vaccine 50 percent glycerin saline with or without saponin. In freeze-dried vaccine the stabilizer must be declared.
- Counter-indications and reactions that may follow vaccination: do not vaccinate pregnant animals. Sometimes severe reactions are observed in goats.
- Instruction: “shake before use” for liquid anthrax spore vaccine.
- Reconstitute the freeze-dried vaccine with physiological saline solution.
- Reconstituted vaccine to be used within two hours or stored at +4°C.
- Storage and shipping: Store the vaccine at +4°C and ship under cold conditions in ice boxes.
- Indications: anthrax spore vaccine, living fulfils the requirements published by WHO in its Technical Report Series No. 361, 1967.

DISTRIBUTION AND SHIPPING

RELEASE FOR DISTRIBUTION

Release the anthrax spore vaccine lot when all the required quality control tests have been performed, summarized, reviewed, and all the official control requirements are satisfied.

SHIPPING

Ship the anthrax spore vaccine under cold conditions over ice in insulated boxes. Do not freeze the liquid vaccine. Freeze-dried vaccine can be shipped in freezers.

Transportation from the manufacturing laboratories to distribution points should be as rapid as possible. Well-packed vials in insulated boxes can be expected to maintain temperatures of 4–15°C for 24 to 48 hours during transport, provided they are not exposed to direct sunlight. Vehicles should go directly from the point of collection to their destination without

long stops en route. Place a temperature indicator in each box during shipment to know the temperature of the vaccine during shipment.

At distribution point, the vaccine should be stored in a cold room at +4°C. Users of the vaccine who do not have adequate storage facilities should obtain it directly from distribution point. In the field, when maintenance of low temperature is impracticable, the vaccine should not be exposed to direct sunlight and utilized within a short time.

STORAGE AND EXPIRY DATE

STORAGE

Store anthrax spore vaccine, living, in a dark and dry place at +4°C in a cold room. Storage at room temperature is not recommended. In field conditions, it is difficult to maintain vaccine at low temperature; all efforts should be made that vaccine is exposed to room temperature for a short period only. During vaccination, store the vaccine in insulated boxes covered with ice. It is necessary to protect the vaccine from direct sunlight.

EXPIRY DATE

Expiry date of the anthrax spore vaccine depends upon the type of vaccine. Generally, liquid vaccine can be expected to retain its potency for a period between six to 12 months from the date of the completion of the potency test at +4°C. There are reports that some liquid anthrax spore vaccines are stable up to 24 months. Stability of freeze-dried vaccine is more than 24 months at +4°C.

INSTRUCTIONS FOR USE OF VACCINE

DESCRIPTION

The vaccine is a suspension of living spores prepared from an uncapsulated strain (34 F 2) of *B. anthracis* preserved in 50 percent glycerine saline.

It has a syrup-like consistency of brownish colour, with a small deposit on the bottom of the vial which is evenly homogenized in the fluid mass when shaken.

USE

It is used to prevent anthrax in cattle, buffaloes, horses, sheep, goats and pigs. It can also be used in camels and elephants.

It should be inoculated subcutaneously: in cattle, buffaloes and horses, in the middle of the neck; in sheep, goat and swine on the inner face of the thigh.

DOSE

Cattle, buffaloes, horses	1.0 ml
Sheep, goats and pigs	0.5 ml

REACTION

The vaccinated animals may have a mild local oedema at the site of inoculation and also a febrile reaction usually lasting for two or three days. In lactating animals there may be a decrease in milk production for two to three days.

IMMUNITY

The immunity is established in ten days following inoculation. The animals are protected against natural infection for about one year.

STORAGE

Stored in a dark and dry place at +4°C, it maintains its potency for 24 months.

PRECAUTIONS

It should not be used by persons who have abrased skin on their hands. After use syringes and needles should be thoroughly sterilized in boiling water for one hour. Do not vaccinate pregnant and young animals under three months of age. Animals suffering from pleuropneumonia should not be vaccinated. Shake the vials before use.

PACKING

The vaccine is issued in 10 and 20 ml vials.

APPENDIXES

Appendix 1
Media

NUTRIENT BROTH

It can be prepared from media available from commercial sources as per manufacturer's instructions or can be prepared in laboratories as follows:

Meat extract (Difco)	3 g
Peptone	10 g
Sodium chloride	5 g
Distilled water	1 000 ml

Weigh all the ingredients in a flask, add 1 000 ml of distilled water and dissolve by boiling in autoclave for 20 minutes. Adjust the pH to 8.2 and autoclave at 110°C for ten minutes to precipitate the salts. Finally adjust the pH to 7.4, filter and distribute as desired and sterilize at 121°C for 30 minutes in the autoclave.

NUTRIENT AGAR

Mix 1.5 to 2 percent of agar with the nutrient broth, melt in autoclave at 110°C for 20 minutes. Adjust the pH to 7.6 when cool. Dispense 5 ml molten agar in tubes for making the slant, 20 ml in tubes for making the stab agar and 120 ml in Roux flasks for production of vaccine. Sterilize by autoclaving at 121°C for 30 minutes. For making the slants, tubes are placed in a slanting position while the medium is hot. Roux flasks are placed on the flat surface of a working bench. When the agar solidifies, incubate at 37°C for 48 hours to detect contamination. Store the tubes at +4°C. Roux flasks are used for production of vaccine immediately.

CASEIN DIGEST AGAR MEDIUM

Tryptic digest of casein	50 g
Yeast extract (Difco)	10 g
Calcium chloride ($CaCl_2$, $6H_2O$)	0.1 g
Ferrous sulphate ($FeSO_4$, $7H_2O$)	0.01 g
Magnesium sulphate ($MgSO_4$, $7H_2O$)	0.05 g
Manganese sulphate ($MnSO_4$, $4H_2O$)	0.03 g
Dipotassium hydrogen phosphate (K_2HPO_4)	5.0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.0 g
Agar	22.0 g
Distilled water	1 000 ml

Weigh the six chemicals and dissolve in distilled water, adjust the pH to 7.6. Add agar and dissolve by autoclaving at 110°C for 30 minutes. Adjust the pH to 7.4 and add the potassium buffer salts. Dissolve and filter the medium. Distribute 120 ml in each Roux flask and sterilize in autoclave at 121°C for 30 minutes. After sterilization, flasks are placed on the table on a flat surface at room temperature. After the solidification of agar, incubate the flasks at 37°C for 48 hours to detect contamination. Examine each flask carefully after incubation and discard the contaminated ones.

SOYBEAN-CASEIN DIGEST MEDIUM

Pancreatic digest of casein	17.0 g
Papaic digest of soybean meal	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g
Distilled water	1 000 ml
Final pH	7.1–7.5

Dissolve the ingredients in warm water, then cool to room temperature. Adjust the pH to 7.6 with 1 N sodium hydroxide. Filter, distribute into suitable vessels and sterilize in autoclave at 121°C for 20 minutes.

FLUID THIOGLYCOLATE MEDIUM

L-cysteine	0.5 g
Sodium chloride	2.5 g
Glucose	5.5 g
Agar	0.75 g
Yeast extract (Difco)	5.0 g
Pancreatic digest of casein	15.0 g
Distilled water	1 000 ml
Sodium thioglycolate	0.5 g
Resazurin sodium (0.1% fresh solution)	1.0 ml
Final pH	7.0–7.2

Grind the first six ingredients in a mortar. Add 200 ml of warm water, stir and transfer to a suitable container, add the remaining distilled water. Dissolve by boiling in water bath, taking special care to ensure complete solution of the L-cysteine. Add sodium thioglycolate, adjust the pH to 7.4, filter and add resazurin solution. Distribute into suitable vessels and sterilize by autoclaving at 121°C for 20 minutes. Cool to 25°C immediately and store at 20–30°C avoiding excessive light. If the uppermost portion of the medium has changed to a pink colour and this exceeds one third of the depths of the medium, it is unsuitable for use but may be restored once by heating in steam. Medium more than three weeks old should not be used.

Appendix 2
Procedure for detection of motility of organisms

The motility of the bacteria can be examined by the following methods:

HANGING DROP METHOD

The motility of bacteria can be observed by examining a hanging drop in which the organisms are able to move about freely. A special glass cavity slide with a hollow depression in the centre is used for this purpose. The method of detection of motility is described below:

- By a match-stick a thin layer of vaseline is placed around the rim of the cavity of the glass slide.
- Place a clean and dry cover slip on a working bench. Transfer a loop of broth culture of bacteria to the centre of the cover slip. In absence of broth culture, emulsify a small amount of culture from solid medium in a drop of broth or normal saline solution taking precautions that the emulsion is not dense.
- Invert the cavity slide over the cover slip and slightly press the slide, so that the glass adheres to the vaseline. Now quickly turn around the slide, so that the cover slip is uppermost. The drop of culture is “hanging” from the cover slip in the centre of the cavity.
- Examine under the microscope with the lower objective and locate the edge of the drop.

- Since excessive illumination renders the organisms invisible, it is necessary to bring down the condenser slightly and partially close the diaphragm.
- Now change to the high-power objective. Focus the edge of the drop and examine by adjusting the condenser and diaphragm.

It is not advisable to use an oil-immersion objective because due to the oil, the cover slip moves during focusing and false motility is produced.

During examination of motility it is essential to distinguish between true motility and Brownian movements. In true motility, organisms change their position in the field and cross the field. The Brownian movement is an oscillatory movement possessed by all small bodies, either living or dead, suspended in fluid. In Brownian movement neither is the position of the organism changed nor do organisms cross the field.

CULTIVATION IN SEMI-SOLID AGAR

In semi-solid agar, motile bacteria “swarm” and give a diffuse spreading growth which can be easily seen by naked eye. Motility thus can be detected more easily than by the “hanging drop” method.

Dissolve 0.2 percent of “Difco” agar in nutrient broth. It is important that the medium should be quite clear and transparent. Dispense 10 ml in test tubes and sterilize at 121°C for 30 minutes. Keep the medium to set in vertical position. Inoculate the culture under test with a straight platinum wire, making a single stab down from the centre of the tube to half the depth of the medium. Examine after incubation at 37°C for 18 hours.

The growth of non-motile bacteria is confined to the stab line and has sharply defined margins, leaving the surrounding medium clearly transparent. Motile bacteria typically give diffuse, hazy growths that spread throughout the medium rendering it slightly opaque.

Appendix 3
Stabilizers for freeze-drying

HORSE SERUM

Collect the horse blood under aseptic conditions and allow it to clot. Store the container at 4°C. Take out the clear serum with a pipette. Filter it by EK Seitz pad and inactivate at 56°C for 30 minutes in a water bath. Store it at 4°C.

Mix an equal volume of sterile inactivated horse serum with spore suspension of *B. anthracis* and freeze-dry.

SKIMMED MILK

Mix an equal volume of 5 percent of sterile skimmed milk with spore suspension of *B. anthracis* and freeze-dry.

SUCROSE-GLUTAMATE-DEXTRAN

Sucrose	5.0 g
Sodium glutamate	1.0 g
Dextran	5.0 g
Physiological saline solution	1 000 ml

Sterilize the solution by filtration. Suspend the spores in the above solution and freeze-dry.

Appendix 4
Procedure for viable spore count

There are many procedures to carry out the viable spore count. The commonly used “pour plate” method is described below.

To avoid the possibilities of error, it is advisable to carry out spore counts in not less than three samples of spore concentrate. Spores have the tendency to clump together and settle to the bottom. These clumps should be dispersed by thorough shaking before drawing samples for counting.

- Put 8 test tubes in a test tube rack, number them and add 9 ml of physiological saline solution to each tube.
- Transfer one ml of spore suspension in the tube No. 1 by a pipette or tuberculin syringe.
- By using a fresh tuberculin syringe or pipette, mix the first dilution several times and transfer 1 ml into the next tube. Make the remaining tenfold dilutions in the same way using a fresh syringe or pipette for each dilution.
- Starting from highest dilution, pipette 1 ml from each dilution of 10^{-8}, 10^{-7} and 10^{-6} into Petri dishes. For each dilution, five Petri dishes of 10 cm diameter are used.
- Now pour into each Petri dish about 20 ml of melted nutrient agar cooled to 45°C. Mix the inoculum immediately with the agar by rotating the plates. Keep the plates at room temperature to allow the agar to set. Incubate the plates at 37°C for 24 hours.

Count the colonies in all five plates of a particular dilution. Multiply the average number per plate by the dilution factor to obtain the viable count per ml in original suspension. Calculate the average count for each dilution from 10^{-6} to 10^{-8}. To obtain the average viable count in original stock suspension, take the average of three dilutions.

Appendix 5
Summary protocol for production and control of anthrax spore vaccine, living

IDENTIFICATION OF FINAL LOT

Name and address of manufacturer _____

Lot number of final product _____

Date of manufacture of final lot _____

Date of filling containers _____

Number of containers and nature _____

Date of last potency test _____

Number of doses in each container _____

Volume of single dose _____

Expiry date _____

STRAINS OF Bacillus anthracis

Strain for vaccine production _____

Name, history and origin _____

Strain for testing _____

Name, history and origin _____

PREPARATION AND CONTROL OF VACCINE SEED-LOTS

Master seed-lot

Date of preparation of master seed-lot _____

Number of passages between master and seed-lot and strain obtained from reference centre _____

Working seed-lot _____

Date of preparation of working seed-lot _____

Number of passages between working _____

seed-lot and production _____

CONTROL OF SEED-LOTS

Tests for bacterial contamination _____

Methods used

Results _____

PREPARATION AND CONTROL OF VACCINE CONCENTRATE

Preparation of vaccine concentrate

Media

Type of medium used _____
Date of preparation _____
Ingredients _____
Tryptic digest of casein _____
Yeast extract _____
Calcium chloride ($CaCl_2$, $6H_2O$) _____
Ferrous sulphate ($FeSO_4$, $7H_2O$) _____
Magnesium sulphate ($MgSO_4$, $7H_2O$) _____
Manganese sulphate ($MgSO_4$, $4H_2O$) _____
Dipotassium hydrogen phosphate (K_2HPO_4) _____
Potassium dihydrogen phosphate (KH_2PO_4) _____
Agar _____
Distilled water _____
pH _____
Number of Roux flasks prepared _____
Sterilization _____
Duration media incubated at 37°C _____
Results of incubation _____

Inoculation

Date _____
Volume of inoculum _____
Number of Roux flasks inoculated _____
Duration of incubation at 37°C _____
Duration of incubation at room temperature _____
Date of checking of sporulation _____

Harvesting

Date _____
Number of Roux flasks harvested _____

Purity test

Date _____
Number of Erlenmeyer flasks containing spore suspension tested _____
Test methods _____
Results _____

Glycerination

Date _____
Weight of empty flask _____

Number of Erlenmeyer flasks containing spore suspension pooled _____

Weight of spore suspension _____

Weight of neutral glycerine added _____

Duration of storage glycerinated _____

suspension at room temperature _____

Control tests on vaccine concentrate

Tests for bacterial contamination

Date _____

Test methods _____

Results _____

Test for number of culturable spores

First spore count _____

Date _____

Results _____

Second spore count _____

Date _____

Results _____

Third spore count _____

Date _____

Result _____

Average spore count _____

Dilution factor _____

Safety test

Animal species used _____

Number of animals _____

Route and dose of inoculation _____

Date of inoculation _____

Date of end of test _____

Number of animals that showed postinoculation reaction _____

Number of animals died _____

Results _____

Immunogenicity test

Animal species used _____

Number of animals _____

Date of inoculation _____

Dose and route of inoculation _____

Number of animals died before challenge _____

Date of challenge _____

Challenge strain used _____

Number of controls _____

Number of vaccinated challenged _____

Dose and route of challenge _____

Number of vaccinated died _____

Number of controls died _____

Results _____

Dilution of vaccine concentrate

Diluent

Type of diluent _____

Date of preparation _____

Weight of glycerine _____

Quantity of saline _____

pH _____

Quantity prepared _____

Sterilization _____

Dilution _____

Date _____

Dilution factor _____

Quantity of vaccine concentrate _____

Quantity of glycerine saline _____

Total quantity of vaccine _____

ADJUVANT

Type _____

Date _____

Concentration of adjuvant used _____

Total quantity of adjuvant added in vaccine _____

FILLING AND CONTAINERS

Liquid vaccine

Date _____

Quantity of containers filled _____

Volume of vaccine per container _____

Freeze-dried vaccine

Date _____
Type of stabilizer _____
Quantity of vaccine mixed with stabilizer _____
Quantity of vaccine filled per container _____
Quantity of container freeze-dried _____

CONTROL TESTS ON FINAL PRODUCT

Inspection of final containers

Date _____
Results _____

Identity test and tests for absence of bacterial contamination

Date _____
Methods _____
Results _____

Test for number of culturable spores

Date _____
Average results of three spore counts _____

Determination of Ph

Date _____
Results _____

Innocuity test

Animal species used _____
Number of animals _____
Route and dose of inoculation _____
Date of inoculation _____
Results _____

Residual moisture test for freeze-dried vaccine

Date _____
Method used _____
Results _____

Stability test

Date _____

Exposure temperature and duration _____

Results _____

Appendix 6
Certifications

INTERNAL CERTIFICATION

This certificate should be issued by the person taking entire responsibility for production of the vaccine.

I certify that lot No. _____of anthrax spore vaccine, living, satisfies Part A of the WHO Requirements for Anthrax Spore Vaccine, Live (Veterinary).

Signature _____

Name _____

Date _____

The protocol must be accompanied by a sample of the label and copy of the leaflet.

RELEASE CERTIFICATION BY NATIONAL CONTROL AUTHORITY FOR EXPORT OF VACCINE

I hereby certify that batch No. _____of anthrax spore vaccine, living, produced by (name of the producer) meets all national requirements as well as part A of the WHO Requirements for Anthrax Spore Vaccine, Live (Veterinary). The date of the last satisfactory potency test carried out by the National Control Authority is _____.

The final lot has been released by us under number _____.

The number appearing on the label of the containers is _____.

Signature _____

Name _____

Date _____

REFERENCES

_____1985. *British Pharmacopoeia* (Veterinary). London, Her Majesty's Stationery Office.

WHO. 1967. *Requirements for anthrax spore vaccine (Live for veterinary use)*. Technical Report Series, 1967, No. 361. (Requirements for biological substances No. 13). Geneva, World Health Organization.

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Appendix 6

COLORADO SERUM COMPANY BIOLOGICAL PRODUCTS		
VACCINE	**DESCRIPTION**	**SPECIES***
Anthrax Spore Vaccine	Non-encapsulated Live Culture	C,S,G,P,H
Bluetongue - Type 10	Type 10 Modified Live Virus	S,G
BOVI-SERA Serum Antibodies	*Actinomyces Pyogenes-Escherichia coli-Mannheimia Haemolytica-Pasteurella, multocida-Salmonella* Typhimurium Antibody, Bovine Origin	C,S
Brucella Abortus Strain - RB-51	Brucella abortus vaccine strain RB-51 Live Culture	C
BVD IBR-PI3	Modified live virus of bovine tissue culture origin, IBR-BVD-PI3	C
Campylobacter Fetus Bacterin - Ovine	Aqueous suspension of inactivated cultures of *Campylobacter fetus* subspecies *jejuni* (type I) and subspecies *intestinalis* (type V) and contains aluminium hydroxide as an adjuvant.	S
CASE-BAC	Detoxified and purified whole culture of Corynebacterium pseudotuberculosis.	S
CASEOUS D-T	Clostridium tetani-perfringens Type D-Corynebacterium pseudotuberculosis bacterin-toxoid	S
Chlamydia Psittaci Bacterin	Aqueous suspension of inactivated cultures of *Chlamydia psittaci*, abortigenic serovar, emulsified with a mineral oil adjuvant.	S
Clostridium Perfringens Types C&D Antitoxin		C,S,G,P
Clostridium Perfringens Types C&D Antitoxin – Equine Origin		C,S,P
Clostridium Perfringens Types C&D Tetanus Toxoid		C,S,P
Clostridium Perfringens Types C&D Toxoid		C,S,P
Ecephalomyelitis Vaccine Eastern&Western	Formalin killed cultures of Eastern and Western Encephalomyelitis viruses	H
Encephalomyelitis Vaccine E&W Tetanus Toxoid	Formalin inactivated cultures of Eastern and Western Encephalomyelitis viruses plus Tetanus Toxoid, detoxified with formalin and moderate heat.	H
ENZABORT		S
Erysipelas Serum Antibodies	Prepared from the blood of horses hyperimmunized with *Erysipelothrix rhusiopathiae.*	P
ESSENTIAL 1	Formalin inactivated *Clostridium haemolyticum*, aluminium hydroxide adsorbed.	C,S,G
ESSENTIAL 2	*Clostridium chauvoei-septicum* Bacterin.	C,S,G
ESSENTIAL 2+P	*Clostridium chauvoei-septicum-Mannheimia haemolytica-Pasteurella*	C,S,G

	multocida Bacterin	
ESSENTIAL 3	*Clostridium perfringens* Types C&D Toxoid	C,S,G,P
ESSENTIAL 3+T	*Clostridium perfringens* Types C&D, Tetanus Toxoid	C,S,G,P
ESSENTIAL 4	*Clostridium chauvoei-septicum novyi-sordellii* Bacterin-Toxoid	C,S,G
LEPTO-5	*Leptospira canicola-grippotyphosa-hardjo icterohaemorrhagiae-pomona* Bacterin	C,P
Mannheimia Haemolytica - Pasteurella Multocida Bacterin	Chemically killed, aluminium hydroxide adsorbed, cultures of *Mannheimia haemolytica*, and *Pasteurella multocida*, bovine isolates.	C,S,G
Normal Serum– Equine Origin	Prepared from the blood of normal healthy horses.	H
Ovine Ecthyma Vaccine (Sore Mouth)	Ovine Ecthyma Live Virus	S,G
PRE-BREED 6	IBR-LEPTO 5	C
PRE-BREED 8	IBR-BVD-PI3-LEPTO 5	C
PULMO-CLEAR [Caprine Serum Fraction, Immunomodulator]	Caprine Serum Fraction	H
Ram Epididymitis Bacterin	An inactivated aqueous culture of *Brucella ovis* isolated from the epididymitis of an infected ram, adsorbed with aluminium hydroxide.	S
RESPIRA 1	Bovine Rhinotracheitis Modified Live Virus Vaccine IBR	C
RESPIRA 3	Bovine Rhinotracheitis-Virus Diarrhea-Parainfluenza $_3$ Modified Live Virus Vaccine IBR-BVD-PI_3	C
RESPIRAGEN SERUM ANTIBODIES	*Actinomyces pyogenes-Mannheimia haemolytica- Pasteurella multocida* Antibody	C,S
Salmonella Dublin-Typhimurium Bacterin	Formalin killed, aluminium hydroxide adsorbed cultures of *Salmonella dublin* and *Salmonella typhimurium*, bovine isolates.	C
Tetanus Antitoxin – Equine Origin		C,S,G,P,H
Tetanus Toxoid - Concentrated		C,S,G,P,H
Tetanus Toxoid - Unconcentrated		C,S,G,P,H
Wart Vaccine	A killed virus of bovine origin.	C
West Nile Virus Antibody	Equine antibodies to WNV.	H
* C cattle; S sheep; G goat; P pig; H horse		

SUMMARY OF CAPITAL COSTS

Item	Cost	Description	Detail
Constructing and equipping manufacturing facility	1,572,000		Table 11.1
Plant commissioning	487,000		Table 11.2
Upgrade animal facility to PC3 for potency testing	150,000	Facility complying with AS, equipped with animal cages, two-way autoclave and access to incinerator.	Not needed if potency testing is outsourced. See discussion.
Total	2,209,000		

Production Costs

Item	Cost		Detail
	Low yield, 100ml pack	High yield, 500 ml pack	
Production	495,630	98,891	Table 11.8
Formulation	56,436	56,409	Table 11.8
Annual recurring costs	139,393	139,393	Table 11.4
Insurance	100,000	100,000	
Total	791,459	394,693	
Cost per dose	1.76	0.88	
Farmer price	2.07	1.03	

Sensitivity Test

	Maximum	Minimum
Production	1,800,856	98,891
Formulation	56,436	56,409
Annual recurring costs	139,393	139,393
Insurance	100,000	100,000
Total	2,096,685	394,693
Cost per dose	4.66	0.88

External manufacturer

	Low yield, 100ml pack	High yield, 500 ml pack	
Production	495,630	98,891	Table 11.8
Formulation	56,436	56,409	Table 11.8
Annual recurring costs	139,393	139,393	Table 11.4
Insurance	0	0	
Total	691,459	294,693	
Cost per dose	1.54	0.65	
Commercial margin	1.02	0.44	
Price to distribution	2.56	1.09	
Farmer price	3.01	1.28	

POSITION	Annual Salary	Super	Other on-costs	Annual cost	Casual load	Daily rate
Manufacturing Manager	85,000	7,650	18,530	111,180	20%	533.7
Quality (Assurance / Quality Control) Manager	80,000	7,200	17,440	104,640	20%	502.3
Technical Officers (up to 3 at various stages of production)	45,000	4,050	9,810	58,860	20%	282.5
Regulatory Affairs	55,000	4,950	11,990	71,940	20%	345.3

TABLE 11.1 CAPITAL COSTS: MANUFACTURING FACILITY

Item			**(2002)**	**Variance**
Premises	**Sole Purpose**	**Multi Purpose**		
Shed	180,000	360,000	60,000	60,000
Cool room (to hold bulk material)	5,000		4,000	1,000
Cool room (to store bulk and finished vaccine)	20,000		20,000	0
Paths and access areas	50,000		50,000	0
Interior lining (freezer panel)	100,000		100,000	0
Air conditioning and treatment	120,000		100,000	20,000
Plans and permits	20,000		20,000	0
Project management	49,500		70,000	0
Total	544,500	724,500	334,000	81,000
Equipment				
Water filter (Milli-Q system)	20,000		12,000	8,000
Balance (for raw materials)	2,500		2,000	500
pH meter	1,500		1,200	300
Stainless steel benches & stools	8,000		3,000	5,000
Computer (SOPs, inventory etc)	10,000		5,000	5,000
Steam (non-attended boiler)	40,000		20,000	20,000
Refrigerator (for seed material)	1,500		1,500	0
Glassware etc	20,000		20,000	0
Total	103,500		64,700	38,800
Laboratory				
Formalin decontamination pass through hatch	25,000		10-50,000	-25,000
Autoclave (two way, accommodating 200L carboys)	600,000		50-600,000	-360,000
Hot air oven (to sterilise glassware)	40,000		10,000	30,000
Benches and stools	5,000		5,000	0
Microscope	12,000		5,000	7,000
Biohazard unit (2.6 m)	15,000		12,000	3,000
Formalin fumigation equipment (to fumigate laboratory)	2,000		1,500	500
Laboratory supplies (pipettes etc)	10,000		3,000	7,000
Peristaltic pumps (deliver inoculum to culture vessels and to fill product)	5,000		4,000	1,000
Incubator, walk-in (to incubate growth of organism)	5,000		2,000	3,000
Special air conditioning (supply Class D air)	5,000		4,000	1,000
Total	724,000		696,500	-332,500
Other Items				
Security	30,000			30,000
Product filling, labelling	50,000			50,000
QC testing equipment/rooms	120,000			120,000
Total All Items	1,572,000		#REF!	#REF!

Table 11.2 Commissioning and Ot

Item	Cost Estimate
Stability test program: Involves storage for 36 months [$10,000], testing at 0, 3, 6, 12, 18, 24, 36 months [7 samplings, 3 batches, viable spore count, pH, appearance, microbial contamination]; potency testing and safety testing at initial and 24 month timepoints (2 x $30,000 + 2 x $8,000 – 3 concurrent tests adds labour only and same PC3 room hire cost).	106,000
Production of stability batches	261,000
Registration (dossier preparation, registration fee, responses to APVMA questions, finalisation of printed labels, receipt of registration certificate)	15,000
Compliance with cGMP	35,000
Bacillus anthracis master seed (34F2) validation	70,000
Total	487,000

her Start-up Costs

	Rationale
	Requirement of registration. Assumes potency tests contracted to AAHL ($76,000 of total). Stability required for a minimum of 3 batches. Test meets APVMA requirements to support 3 year shelf life. Assumptions: 3 batches of 400 flasks packed in 100ml and 500ml units, 50% batch success rate (Table 11.8)
	Product must be registered in order to be sold.
	Validation of processes and procedures, writing of manufacturing documents, site master plan, SOPs etc. Claxton estimate of $20,000 now insufficient to meet new cGMP.
	Compliance with 9CFR (safety, potency and other tests). Includes MLD determination + vaccine potency test. Assumes contracted to AAHL (60,000 of total).

TABLE 11.3 CAPITAL COSTS: LABOUR

Time (days) Description

Initial plus annual validation, maintenance, calibration

10 Equipment (Autoclave Oven sterilisers Laminar flow cabinets Balances pH meter Thermometers Incubators Cold storage)
Decontamination process (environmental sampling)
HEPA filters (external provider)
Air pressure gradients and flow (external provider)
Filling process

Seed lots

16 Master Seed Preparation
Plate parent culture
Incubate
Transfer to agar slants and incubate
Transfer to Roux flasks and incubate
Harvest, purity check, pool, label, freeze dry, store
[9 days culture + 7 days purity]

2 Working Seed Preparation
Reconstitute master seed
Inoculate agar slants
Harvest, purity check, label, store at 4^0C for up to 6 months

7 Control of seed lots I
Bacterial contamination
Morphology (G stain plus microscopy)
Culture
Nutrient agar plate (24-48h)
Erlenmeyer flask with nutrient broth (to 7 d)
Erlenmeyer flask with thioglycolate fluid (to 7 d)
Motility

31 Control of seed lots II (tests undertaken after production of vaccine)

Safety (sheep) (observe for 14 days)
Potency (guinea pigs) (observe for 31 days)

TABLE 11.4 ANNUAL RECURRING COSTS

COST ITEM	2005		Claxton 2002	REASON FOR VARIANCE
Item	**Cost**	**Detail**		**Detail**
Regulatory Related				
Staff training	3,200	Time for preparation and delivery of training by Quality Manager.	2,000	Time for preparation and delivery of training by Quality Manager. Additional 12 hours.
Documentation review & reissue	2,600	Documentation revision demands imposed by 2005 cGMP.	1,000	Increased documentation revision demands imposed by 2005 cGMP. Additional 16 hours.
APVMA Annual GMP inspection	1,000	Covers cost of audit by an accredited GMP auditor	1,000	The cost covers the cost of having an audit by an accredited GMP auditor
APVMA Manufacturers Licence Fee	1,500	Fee for Category 1 facility (immunobiologicals). May be paid in 4 yearly instalments.	1,000	The licence fee for a Category 1 facility (immunobiologicals) is a one-off fee of $6,000 which can be paid in 4 yearly instalments.
APVMA Product Levy	4,050	Levy 0.9% of sales	1,000	Levy based on sales
AQIS Approved Premises permit	1,000		1,000	
AQIS in vivo permits	680		360	Change in AQIS fee structure
Preparation for APVMA audits – labour	3,200		2,400	Increased documentation demands. Additional 8 hours for Quality Manager.
Preparation of AQIS Permit applications	600		600	
Regulatory Sub Total		17,830		
Equipment Validations				
HEPA filters and Laminar Flow units	2,500		2,500	
Autoclave	1,500		1,500	
Sterilizing oven	500		500	
Other	500		500	
Air flow validation	1,500	Requirement of PC-3 facilities		Requirement of PC-3 facilities
Calibration of scales	500	GMP requirement		GMP requirement
Security	5,000	Additional security anticipated when AG report issued.	5,000	Additional security anticipated when AG report issued.
Health monitoring of staff	1,000	Periodic health monitoring recommended by PC-3 requirements		Periodic health monitoring recommended by PC-3 requirements
Equipment replacement	1,000	Assumed each HEPA filter needs to be replaced every 5 years.		Assumed each HEPA filter needs to be replaced every 5 years.
Storage				
Retention sample storage	1,200	Estimate: $3/day		
Master seed storage (at two sites)	5,000	Estimate: $12/day		
Equipment Maintenance				
Scheduled and as needed	5,000			
Depreciatiion				
Plant	13,613	Rate 2.5%		
Equipment	82,750	Rate 10%		
Total	139,393			

TABLE 11.5 MANUFACTURING CAMPAIGN LABOUR COSTS
(based on Misra 1991)

VACCINE MANUFACTURE (typical campaign) (71.3-103.3 days)

STEP	TIME (days)	DESCRIPTION
DECONTAMINATION (5.4 days)		
	4	Clean
	1	Fumigate
	0.2	Environmental sampling (microbial: surfaces, walls, benches etc; temperature and humidity). Microbiology laboratory
	0.2	Document and review results
MATERIALS AND MEDIA PREPARATION (10 days)		
	10	Master Manufacturing Formula, Batch Manufacturing Instructions, other documents
		Glassware clean and autoclave
		Clothing clean and autoclave
		Nutrient agar, casein digest agar, fluid thioglycolate, soybean-casein digest, motility agar.
		Glycerol, saline, saponin, water
		Packaging materials
		Filling equipment
		Labelling (including leaflets)
SEED LOTS (8 days)		
	1	<u>Working Seed Preparation</u>
		Reconstitute master seed
		Inoculate agar slants
		Harvest, purity check, label, store at 4^0C for up to 6 months
	7	<u>Control of seed lots</u>
		Bacterial contamination
		Morphology (G stain plus microscopy)
		Culture
		nutrient agar plate (24-48h)
		Erlenmeyer flask with nutrient broth (to 7 d)
		Erlenmeyer flask with thioglycolate fluid (to 7 d)
		Motility
VACCINE CONCENTRATE (30.5-62.5 days)		
(Each Roux flask produces around 2,000 vaccine cattle doses)		
1	2	Test sterility of Roux flasks: Incubate 48 hours
2		Prepare working seed (see above)
3	3 to 10	Inoculate Roux flasks and incubate (3 days at 37^0C thence up 7 days at RT, checking degree of sporulation)
4	2 to 6	Harvest when at least 90% sporulation (10 Roux flasks pooled in 1 Erlenmeyer flask)
5	0.5	Purity test (incubate sample from each flask overnight and check with G stain and motility)
6	0.5	Glycerination (1 part spore suspension diluted in 2 parts glycerol)
7	0.5	Purity test

8	1 to 22	Remove vegetative bacteria (heat at 65^0C for 1 hour, followed by storage at 4^0C for up to 21 days)
9	21	Control tests	
		Contamination (morphology, culture, motility) (7 days)	
		Spore count (2 days)	
		Safety (14 days)	
		Potency (assumption: not required as routine test)	

DILUTION OF VACCINE CONCENTRATE (10 days)

1	2	Dilute concentrate with neutral 50% glycerine saline	
2		Add adjuvant (0.1% saponin – 1mg/ml)	
3		Mixing	
4		Filling	
5		Closing	
6		Labelling	
7		Retention samples	
8	9	Control tests	
		Inspection	
		Contamination	
			Morphology
			Culture
			Motility
		Identity	
		Spore count	
		pH	
9		Storage	

DECONTAMINATION (6.4 days)

1	1	Waste disposal
2	4	Clean
3	1	Fumigate
4	0.2	Environmental sampling (microbial: surfaces, walls, benches etc; temperature and humidity) Microbiology laboratory
5	0.2	Document and review results

DOCUMENTATION

As required by cGMP and other regulations.
Responsibility of Quality Manager

COMMENTS: If Roux flasks are unavailable it may be possible to develop a suitable method with Erlenmeyer flasks. This may form part of a continous improvement exercise as encouraged by GMP.

SUMMARY		
Bulk Ag		Decontamination (5.4), Materials and media preparation (10), Seed lots (8),
	53	Vaccine concentrate (2+7+4+0.5+0.5+0.5+5+10=29.5)
Formulation	16.4	Dilution (10), decontamination (6.4)

TABLE 11.6 MANUFACTURING CAMPAIGN: BULK ANTIGEN MATERIAL COSTS

BULK ANTIGEN PRODUCTION	COST ESTIMATE ($) (Claxton 2002)
Materials	
Paraformaldehyde	30
Vircon S	75
Environmental plates	10
Medium	Range 236-948
Flasks	Range 760-3,040
Pipettes	Range 5-20
Glycerol	Range 70-280
Operating Costs (Per batch)	
Power, water, gas	2,100
Maintenance	800
Laboratory clothing	
Covershoes	Range 10-30
Masks	Range 10-30
Gloves	Range 100-300
Laboratory coats	Range 20-40
Sterile CRG coveralls	Range 150-450
Sterile CRG hoods	Range 30-90
Sterile overshoes	Range 100-300
Labour	(@ $200 per person day)
Line clearance / equipment preparation / facility cleanup	600
Fumigation	100
Media preparation	400-600
Preincubation & inspection	50-80
Prepare working seed	250-350
Inoculation	400-1200
Incubation (5 days) – check laboratory functioning	200
Harvest & sampling	400-1200
Purity	150
Spore count	250
Facility cleanup	600
Fumigation	100
Repeat cleanup	400
Repeat Fumigation	100
Documentation: Antigen preparation	100
Documentation: Facility/equipment functioning	100
Labour Subtotals	
100 flasks	4200
200 flasks	4915
400 flasks	6130

TABLE 11.7 MANUFACTURING CAMPAIGN: VACCINE FORMULATION MATERIAL

VACCINE FORMULATION (150,000 dose batch)	**COST ESTIMATE ($) (Claxton 2002)**
Materials	
Paraformaldehyde	30
Vircon S	75
Environmental plates	5
Glycerol	Range 1,736-2,475
Packaging materials – 100 dose packs	
100 ml pillow packs	630
Rubber stoppers	120
Caps	65
Labels	60
Leaflets	60
Packaging materials – 500 dose packs	
500 ml pillow packs	126
Rubber stoppers	24
Caps	13
Labels	12
Leaflets	12
OPERATING COSTS (per batch)	
Power, water, gas	630
Maintenance	240
LABORATORY CLOTHING	
Covershoes	6
Masks	6
Gloves	60
Laboratory coats	8
Sterile CRG coveralls	90
Sterile CRG hoods	18
Sterile overshoes	60
LABOUR	(@ $200 per person per day)
Line clearance / equipment preparation / facility cleanup	600
Fumigation	100
Media preparation	150
Blending	70
100ml packs: Filling, Stoppering, Capping	300
Labelling	75
500ml packs: Filling, Stoppering, Capping	150
Labelling	35
QC TESTING	
Sampling	10
Purity	150
Spore count	250
DECONTAMINATION	
Facility cleanup	600

Fumigation	100
Repeat cleanup	400
Repeat fumigation	100
DOCUMENTATION	
Batch preparation	100
Facility, equipment functioning	100
Labour Subtotals	
100ml packs	3105
500ml packs	2915

	2002 COST CALCULATIONS (Claxton 2002)						
	Number of flasks	100	100	200	200	400	400
	Yield per ml bulk antigen (spore count)	1.0E+08	5.0E+08	1.0E+08	5.0E+08	1.0E+08	5.0E+08
	Bulk antigen volume (L)	1.5	1.5	3.0	3.0	6.0	6.0
	Total bulk antigen (spore count)	1.5E+11	7.5E+11	3.0E+11	1.5E+12	6.0E+11	3.0E+12
	Bulk antigen doses (@ 1.5*10^7 spores/dose)	10,000	50,119	20,000	100,237	40,000	200,475
	Batch cost ($): TOTAL	**8,706**	**8,706**	**10,814**	**10,814**	**14,673**	**14,673**
	Batch cost ($): labour	4,200	4,200	4,915	4,915	6,130	6,130
	Batch cost ($): materials, power, gas, water, maintenance, clothes	4,506	4,506	5,899	5,899	8,543	8,543
	Batch cost ($): other costs						
	Cost per 150,000 doses ($)	130,590	26,056	81,105	16,183	55,024	10,979
	Formulation Cost 150,000 doses (100ml pack) ($) TOTAL	**7,104**	**7,843**	**7,104**	**7,843**	**7,104**	**7,843**
	Formulation cost ($): labour	3,105	3,105	3,105	3,105	3,105	3,105
	Formulation cost ($): materials, packaging, power, gas, water, maintenance, clothes	3,999	4,738	3,999	4,738	3,999	4,738
	Formulation cost ($): other costs						
	Finished product cost (150,000 doses) ($)	137,694	33,899	88,209	24,026	62,128	18,822
	Cost per 1ml dose (100ml pack) ($)	0.92	0.23	0.59	0.16	0.41	0.13
	Formulation Cost 150,000 doses (500ml pack) ($) TOTAL	**6,166**	**6,905**	**6,166**	**6,905**	**6,166**	**6,905**
	Formulation cost ($): labour	2,915	2,915	2,915	2,915	2,915	2,915
	Formulation cost ($): materials, packaging, power, gas, water, maintenance, clothes	3,251	3,990	3,251	3,990	3,251	3,990
	Formulation cost ($): other costs						
	Finished product cost (150,000 doses) ($)	136,756	32,961	87,271	23,088	61,190	17,884
	Cost per 1ml dose (500ml pack) ($)	0.91	0.22	0.58	0.15	0.41	0.12
	Bulk antigen batch success rate	**1.00**					
	2005 COST CALCULATIONS						
	Number of flasks	100	100	200	200	400	400
	Yield per ml bulk antigen (spore count)	1.0E+08	5.0E+08	1.0E+08	5.0E+08	1.0E+08	5.0E+08
	Bulk antigen volume (L)	1.5	1.5	3.0	3.0	6.0	6.0
	Total bulk antigen (spore count)	1.5E+11	7.5E+11	3.0E+11	1.5E+12	6.0E+11	3.0E+12
	Bulk antigen doses (@ 1.5*10^7 spores/dose)	10,000	50,119	20,000	100,237	40,000	200,475
	Batch cost ($): TOTAL	**40,019**	**40,019**	**41,412**	**41,412**	**44,056**	**44,056**
	Batch cost ($): labour	14,974	14,974	14,974	14,974	14,974	14,974
	Batch cost ($): materials, power, gas, water, maintenance, clothes, environmental monitoring	9,506	9,506	10,899	10,899	13,543	13,543
	Batch cost ($): cGMP QA/QC and mfg mgr	15,539	15,539	15,539	15,539	15,539	15,539
	Cost per 150,000 doses ($)	600,285	119,773	310,590	61,971	165,210	32,964
	Formulation Cost 150,000 doses (100ml pack) ($) TOTAL	**18,812**	**19,551**	**18,812**	**19,551**	**18,812**	**19,551**
	Formulation cost ($): labour	4,633	4,633	4,633	4,633	4,633	4,633
	Formulation cost ($): materials, packaging, power, gas, water, maintenance, clothes, environmental monitoring	8,999	9,738	8,999	9,738	8,999	9,738
	Formulation cost ($): cGMP QA/QC and mfg mgr	5,180	5,180	5,180	5,180	5,180	5,180
	Finished product cost (150,000 doses) ($)	619,097	139,324	329,402	81,522	184,022	52,515
	Cost per 1ml dose (100ml pack) ($)	4.13	0.93	2.20	0.54	1.23	0.35
	Formulation Cost 150,000 doses (500ml pack) ($) TOTAL	**18,064**	**18,803**	**18,064**	**18,803**	**18,064**	**18,803**
	Formulation cost ($): labour	4,633	4,633	4,633	4,633	4,633	4,633
	Formulation cost ($): materials, packaging, power, gas, water, maintenance, clothes, environmental monitoring	8,251	8,990	8,251	8,990	8,251	8,990
	Formulation cost ($): cGMP QA/QC and mfg mgr	5,180	5,180	5,180	5,180	5,180	5,180
	Finished product cost (150,000 doses) ($)	618,349	138,576	328,654	80,774	183,274	51,767
	Cost per 1ml dose (500ml pack) ($)	4.12	0.92	2.19	0.54	1.22	0.35
	Bulk antigen batch success rate	**1.00**					
	Changes: 2005 compared with Claxton (2002)						
	Labour: daily rates of pay increased						
	Labour: number of days per stage increased (based on manufacturing methods of FAO 1991 and requirements of PC3 and cGMP)						
	cGMP: increased burden of monitoring and documentation of every manufacturing step now required by GMP license						
	Environmental monitoring: adds approximately $10,000 per campaign, required by cGMP and PC3 standard						

TABLE 11.9 SUMMARY OF MANUFACTURING CAMPAIGN COST

ITEM	COST	DESCRIPTION
Material costs: 100 flasks, 100ml packs	4,635	Tables 11.6 & 11.7. From Claxton 2002
Material costs: 100 flasks, 500ml packs	3,887	Tables 11.6 & 11.7. From Claxton 2002
Material costs: 400 flasks, 100ml packs	8,672	Tables 11.6 & 11.7. From Claxton 2002
Material costs: 400 flasks, 500ml packs	7,924	Tables 11.6 & 11.7. From Claxton 2002
Utilities	2,730	From Claxton 2002
Labour costs (from initial cleanup to completion of batch records).		Table 11.5. Technical officers: 71.3 to 103.3 days. Quality Manager: 20-30 days. Production Manager: 20-30 days