Determining the feasibility of developing an ovine Salmonella vaccine

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Abstract

This project assessed the economic feasibility of developing a Salmonella vaccine for use in Australian sheep. Outputs included a literature review and a benefit-cost analysis. Vaccination is economically viable, based on a positive NPV meaning that the present value of returns over 10 years is greater than the present value of costs. The estimated benefit-cost ratio for Salmonella vaccination is 1:1.13 meaning that every $1.00 invested in the program is expected to return $1.13. While the economic return is positive, the relatively small magnitude of the BCR may lead to questions concerning whether further work should be done on developing a Salmonella vaccine. However, vaccination is considered to have very important intangible benefits, particularly in minimising the risk of outbreaks of salmonellosis sufficiently severe to lead to public and political pressure to close the export industry. The combination of a positive economic impact and intangible benefits provide strong support for further research to develop and register a vaccine against Salmonella in Australian sheep that can be delivered orally through drinking water.

Executive summary

This project was initiated to assess the economic feasibility of developing a Salmonella vaccine for use in Australian sheep, incorporating recent developments in vaccine technology and oral delivery via drinking water. The objectives of the project were to:

1. Complete a literature review of salmonellosis in sheep with particular reference to the livestock export program.
2. Complete a review of Salmonella vaccines.
3. Complete a benefit-cost model relating to use of Salmonella vaccination in sheep intended for live export from Australia.
4. Discuss the findings of the model including consideration of feasibility of developing a commercial vaccine for use in live export sheep and non-economic benefits that may be associated with the application of a Salmonella vaccine in live export sheep.

This report describes current knowledge concerning salmonellosis in live export sheep and about vaccines that are either available or under development as options for reducing the occurrence and severity of salmonellosis. A DNA adenine methylase live attenuated vaccine has been identified that has genuine potential to offer rapid onset of effective heterologous and homologous immunity following oral administration to sheep in the assembly feedlot.

A benefit-cost approach was used to assess losses caused by salmonellosis and the costs and effectiveness of vaccination. The findings of economic modelling indicate that vaccination is economically viable. This conclusion is based on the finding of a positive NPV meaning that the present value of returns over 10 years is greater than the present value of costs over the same time frame. The same output can be expressed as a benefit/cost ratio (BCR) which is simply the ratio of the present value of benefits to the present value of costs. The estimated BCR for Salmonella vaccination is 1:1.13 meaning that for every $1.00 invested in the program the expected return would be $1.13. Sensitivity and breakeven analyses were used to identify and characterise influential input parameters.

Major intangibles associated with the impact of Salmonella vaccination were identified as: 1) those relating to public perceptions about the livestock export trade and additional animal welfare benefits through reduced morbidity; and 2) improved performance associated with a reduction in Salmonella exposure and infection. The impact of intangibles is considered to add weight to an already positive economic result and strengthen the case in favour of development of a Salmonella vaccination strategy.

The major beneficiaries of an effective Salmonella program are;

1. Those industry operators directly involved in the live export trade
2. The broader Australian sheep industry through the impact of the live sheep trade on general domestic sheep prices and;
3. The Australian public through the ongoing strength and sustainability of the sheep industry.

The report supports a recommendation to proceed with research and development of a vaccine against Salmonella that can be delivered orally through drinking water.

Contents

1 Background

The livestock export industry has previously assessed the business case for the development of a vaccine for Salmonella in sheep, concluding that vaccine development could not be justified on an economic basis.

However, during the last 10 years there has been significant development in the area of Salmonella vaccines with the advent of DNA adenine methylase live attenuated vaccines. Also, the number of sheep under intensive management has increased in Australia in the last 10 years with the introduction of initiatives such as lamb feedlotting.

Effective vaccination may offer a cost effective means of reducing salmonellosis in the live sheep trade and has potential applications in other intensively managed sheep enterprises. This project was initiated to analyse the viability of developing a Salmonella vaccine for use in Australian sheep. This assessment will take into account the commercial viability, and also consider the non-cash benefits that the industry could derive from vaccine development.

2 Project objectives

1. Complete a review of previous relevant work undertaken by the livestock export program and of scientific literature relating to salmonellosis in sheep with particular reference to the export program.
2. Complete a review of Salmonella vaccines including mechanisms of action, route of administration, time to onset of immunity, and efficacy as it relates to challenge by homologous and heterologous Salmonella serotypes.
3. Complete a benefit-cost model relating to use of Salmonella vaccination in sheep intended for live export from Australia including sensitivity analysis assessing impact of input parameters and assumptions.
4. Discuss the findings of the model including consideration of feasibility of developing a commercial vaccine for use in live export sheep and non-economic benefits that may be associated with the application of a Salmonella vaccine in live export sheep.

3 Methodology

3.1 Literature review and information gathering

This component of activity will cover a formal review of scientific information and sourcing of grey and informal information relevant to the objectives with particular focus on the following areas:

a. Literature review of vaccine development, usage patterns, efficacy, and economics, for vaccines used to prevent salmonellosis in animals, particularly sheep and cattle.
b. Live export trade statistics
c. Morbidity and mortality associated with salmonellosis and related conditions in live export sheep, including incidence, management/treatment/prevention, prognosis and impact on delivered numbers of sheep and value.
d. Non-cash benefits that may be associated with use of vaccination

e. Economics of vaccine development, production, quality assurance and marketing, as well as related issues including registration requirements, environmental impact, social, political and welfare implications etc.
f. Intellectual property issues associated with particular vaccine technology will need to be resolved if a commercial vaccine is to be developed. One of the investigators (J House) was involved in research at University of California in developing salmonellosis vaccines. Estimates will be sought for costs of incorporating University of California IP in commercial vaccine development.

3.2 Development of economic model

Information sourced from the first activity component was used to develop parameters and scenarios in the following areas:

a. Estimates were made of the size of the potential market for the vaccine, and usage patterns as well as the possible impact of vaccine use on morbidity and mortality. Sensitivity analyses were used to identify key parameters and information gaps of importance to the decision making process.
b. A spreadsheet model was used to represent the development, registration, production and marketing process and the cost per unit of product under different production scenarios.
c. The above components were combined in an economic analysis using a break-even approach, to determine the per-unit price required to break even financially for a range of different parameter values concentrating on market share, vaccine efficacy (morbidity and mortality reduction), and time period before achieving profitability. Since registration of a new product is typically associated with up-front costs while income streams develop over time, parameters such as net present value were used when comparing different scenarios.

3.3 Consultation

Consultation was undertaken with industry stakeholders involved in a range of areas including pharmaceutical product development, sheep consultancy, producers and professionals active in the livestock export trade, and business management for feedback on assumptions and parameters considered for inclusion in the economic analysis.

3.4 Preparation of reports

A draft report has been produced for MLA for circulation to industry stakeholders for consideration and comment. Feedback has been incorporated into the final report.

4 Results and discussion

4.1 Literature review

4.1.1 Industry statistics

Over the last 4 years the annual number of sheep exported from Australia has ranged from 3.7 to 4.2 million with an export value of 280 – 321 million dollars. Live sheep export reflects approximately 12 % of the total sheep and lamb turn-off [1].

The majority of sheep are exported from Fremantle reflecting the lower sheep and shipping costs associated with export from Western Australia. Sheep supply and cost are important variables in the export trade and are impacted by: when and where sheep are sourced, the interval between purchase and assembly, and occasionally on the duration of the assembly period. This is pertinent to the potential implementation of a vaccination strategy as sheep supply has the potential to influence the logistics of vaccine delivery. When supply is limited more sheep are sourced from eastern Australia, sheep are stockpiled for coming orders, and sheep specifications are adjusted to fit with available stock. In 2007 there were 2.7 million sheep exported from Fremantle (Western Australia), 521,000 from Adelaide (South Australia) and 491,000 exported from Portland (Victoria)[2].

4.1.2 Morbidity and mortality

There is little data available regarding the incidence of disease morbidity in the live sheep trade. This reflects the difficulties associated with measuring morbidity. Conversely there have been a number of investigations into causes of mortality. These studies have identified the causes of mortality and factors that influence mortality risk. A progressive reduction has been observed in mortality since the industry began in the 1970s when mortality rates were as high as 4%. More recently the annual mortality rate has been around 1%. Factors that influence mortality include time of year, port of loading, and source, line and class of sheep[3-6].

Mortality investigations conducted in the 1980s and early 1990s demonstrated that inanition and salmonellosis accounted for the majority (60-75%) of the mortalities during the live export process[7, 8]. Salmonellosis and inanition were observed to still be the most common causes of mortality during the LIVE.123 mortality investigation conducted from 2006 to 2008. Salmonellosis alone accounted for 34.4% of mortality, inanition 23.9%, and a combination of salmonellosis and inanition 18.2%.[3] Inanition is defined as the exhausted state due to prolonged under-nutrition or starvation.[9] In the context of live export sheep a proportion of sheep have been observed not to eat the pelleted ration. Prolonged anorexia leads to inanition. A confounding variable in the live export industry is the influence of disease on appetite. Anorexia is a feature of salmonellosis so when a sheep dies with signs of salmonellosis and reduced gut fill it is not possible to tell if the salmonellosis preceded or followed the anorexia. In previous mortality investigations sheep that had signs of salmonellosis and inanition were considered to have died from inanition hence earlier reports suggest that a greater proportion of sheep die from inanition. In the more recent investigation sheep with gross pathological changes consistent with salmonellosis that had low rumen solids were classified as having salmonellosis and inanition.[5]

Each export shipment can be divided into three stages; loading, voyage and discharge. The discharge stage begins on arrival at the first port of unloading. Norris et al. (1989) described changes in mortality rate over time for several shipments and demonstrated that the number of mortalities occurring at different stages was dependent on the disease processes which were occurring.[10] Richards et.al (1989) suggested that salmonellosis was responsible for the majority of deaths early in the export process while a significant number of deaths later in the voyage were due to inanition. [7]

Historically, sheep exported from the eastern ports (Adelaide and Portland) have suffered higher mortality rates than those exported from Fremantle. Mortality data collected in 2002 showed that sheep exported from Portland, were more than twice as likely (relative risk = 2.4) to die during the voyage when compared with sheep exported from Fremantle. Salmonellosis was noted to be a problem in Portland assembly depots during this time.[5] The number of sheep exported from Portland declined over the following years and the problems with salmonellosis subsided. During the LIVE.123 study conducted between 2006 and 2008 it was observed that the proportion of sheep loaded from Portland dying during the voyage was lower than that for sheep loaded from Fremantle and Adelaide. During this period there was a low incidence of salmonellosis in Portland and a higher incidence in Western Australian assembly depots during the months of July to October.

Mortality rates have been higher in the second half of the year, particularly in heavier wethers and rams. It has been hypothesised that the change in season alters sheep metabolism favouring the development of inanition in older heavier wethers in the latter half of the year.[4, 11] Different lines of sheep have also been observed to have variable rates of mortality. Norris et al. (1989) reported 54% of all deaths were in 25% of lines,[6] Higgs et al. (1999) observed 50 % in 14.2% [12] and Makin et al. (2008) 72% of mortality in 16% of lines.[3] Sixty five percent of consignments do not experience any mortality.[3] Sheep from some farms have also been observed to have an increased recurrent risk of mortality. [12] All of these observations support the hypothesis that there may be factors operating at the farm-level that influence mortality risk in export sheep. Attempts to identify farm-level factors have not been successful to date.

4.1.3 Salmonella in the live sheep trade

Salmonellosis and inanition account for approximately 75% of the mortality in the live sheep trade.[3] These conditions may be observed independently or together; however, the two conditions do appear to be closely linked with anorexic sheep succumbing to salmonellosis and conversely sheep with salmonellosis becoming anorexic.

There are over 2,200 reported serotypes of Salmonella and all are potentially capable of causing disease. Over the last 30 years *S. enterica* serovar Typhimurium, *S. enterica* serovar Bovismorbificans, *S. enterica* serovar Havana, and *S. enterica* serovar Anatum have been the predominant serotypes associated with disease in the live sheep export trade.[13]

The prevalence of sheep shedding Salmonella on entry to export assembly depots is less than 1%.[14] Export assembly depot receival and load-out yards are frequently contaminated with Salmonella providing a source of Salmonella challenge for sheep entering these facilities.[3] The outcome of the host-pathogen interaction is variable ranging from resistance to infection to acute, fulminant bacteraemia, endotoxaemia, and death. Salmonellosis is the most common disease problem causing mortality in sheep during the assembly period. Normally the prevalence of disease is low with the incidence increasing during the latter phase of the assembly period. Average assembly

mortality is 5 per 10,000 sheep. Sporadic outbreaks of salmonellosis occur intermittently in assembly depots, resulting in up to 100 deaths per 10,000 sheep.[3, 15] Twenty percent of assemblies may experience an outbreak of salmonellosis during problem years.[15] The frequency of assembly salmonellosis has been associated with higher sheep throughput from May to October. More (2002) reported increased mortality on board ships during the first two weeks of the voyage which he hypothesised reflected a carry-over effect of Salmonella infection incurred during the assembly period.[13] It has also been observed that sheep assembled in paddocks are more likely to experience a Salmonella outbreak than sheep assembled in sheds. However, similar outbreaks have been observed in sheep assembled in sheds.[3]

Exposure of sheep to Salmonella is common in assembly depots.[3, 13] However, the actual effect on individual sheep may vary depending on the challenge dose, pathogen virulence and host immunity. Clinical signs of salmonellosis in sheep include fever, anorexia, diarrhoea, and depressed mentation. Anorexia and fever are the first signs appearing within 48 hours of Salmonella challenge. With severe disease, animals rapidly become emaciated due to prolonged anorexia and associated catabolism induced by the disease state.

There are a large number of causal factors that may influence the outcome of host-pathogen interactions and many of these factors are likely to be dynamic or capable of rapid and frequent change both within and between animals and lines. Variable host immunity may reflect differences in previous Salmonella exposure and presence/severity of stressors including periods of feed deprivation prior to arrival. Variability also occurs in relation to the magnitude of the Salmonella challenge and the virulence of Salmonella in the assembly depots reflecting the influence of sheep throughput, and of environmental conditions on Salmonella proliferation, virulence, and persistence in the environment.[3]

Salmonella infections are most commonly acquired through faecal-oral contamination. Intranasal, conjunctival, and aerosol transmission may also occur.[16-18] Conjunctival Salmonella challenge has been utilised for experimental challenge studies in sheep[19] and may be relevant to sheep passing through Salmonella-contaminated yards. Infection of sheep by *S. enterica* serovar Typhimurium and *S. enterica* serovar Bovismorbificans via yarding sheep in contaminated yards has previously been demonstrated in New Zealand.[20]

The number of Salmonella required to produce clinical disease is dependent on the virulence of the serotype and immunity of the host. The challenge dose utilised to induce disease and mortality in experimental studies is in the range of 10^9 - 10^{11} Salmonella.[21-23] When immunity is compromised by concurrent disease, or physiological or dietary stress, the infectious dose may be several hundred Salmonella organisms.[24] Feed deprivation is inherent in the on-farm processes required for preparing sheep for sale — mustering, yarding, drafting, holding, curfew and transport. Feed deprivation is likely to contribute to an increased susceptibility to Salmonella challenge.

Inherent in the assembly process is a change in diet from pasture to pellets. The growth of Salmonella in the rumen following ingestion is influenced by dietary intake before and after the organisms are ingested.[25] The growth of Salmonella in the rumen is inhibited by high concentrations of volatile fatty acids and a low rumen pH (normal is 5.5-6.5).[26, 27]

When sheep fail to eat, production of volatile fatty acids is reduced and rumen pH in anorexic sheep therefore rises and may approach 7 or 7.5. Reduced feed intake either through interruption of feeding for one or more days (during travel for example), or through anorexia may therefore result in an increase in numbers of Salmonella in the rumen. In contrast, Salmonella disappear rapidly from the rumen of regularly fed ruminants.[25] In addition to inhibiting bacterial replication, volatile fatty acids can also reduce Salmonella virulence.

The biochemical explanation for these effects is complex and some of the effects are actually dependent on the type of fatty acids produced. For example, butyrate and propionate cause suppression of Salmonella invasion of epithelial cells *in vitro*, and yet acetate does not suppress cell invasion. Lawhon et al (2002) explained this effect on the basis of changes in Salmonella pathogenicity island (SP-1) expression. [29] It has been found that SPI-1 contains the Salmonella virulence genes arranged in operons required to invade epithelial host cells during early stages of infection. These genes are transcriptionally regulated by the HilA protein, encoded by a gene of the SPI-1 pathogenic island. [30] Butyrate reduces HilA and some of the genes under its control. [31] Inhibition of HilA expression by butyric acid is pH dependent, and at pH 7 there is nearly no influence on HilA expression. [32] Inhibition is favoured by a more acidic pH. Reduction in feed intake and the consequent rise in rumen pH is associated with loss of inhibition of these regulators and creates an environment that favours rapid proliferation of Salmonella.

Feeding after a period of starvation is also associated with multiplication of Salmonella.[33, 34] Dietary changes that result in clinical or subclinical ruminal acidosis may increase the risk of salmonellosis because rumen acidosis results in disruption of normal fermentation and the production of lactate. [26] Lactate is a stronger acid than the other volatile fatty acids (acetate, proprionate, and butyrate), therefore it is more dissociated than the weaker acids at an equivalent pH. Volatile fatty acids only diffuse across the bacterial cell membrane in the un-dissociated form, [28] and dissociate within the bacterial cell. In its dissociated form lactate is unable to diffuse across the bacterial cell membrane and therefore any beneficial impacts of intra-cellular fatty acids are lost. The lactate acidosis also favours the less fastidious Salmonella in contrast to other rumen micro-organisms and Salmonella can then multiply rapidly using the available substrate. [26] In addition, ruminants with ruminal acidosis are often anorexic for variable periods reflecting systemic endotoxaemia and acidosis. Anorexic individuals recovering from ruminal acidosis may then incur a rise in rumen pH as a consequence of anorexia and the buffering affect of saliva which in ruminants is high in bicarbonate. These changes illustrate some of the complexities in understanding factors that influence Salmonella proliferation and virulence and attempt to explain how Salmonella can proliferate under conditions of elevated rumen pH and in cases where rumen pH may be reduced, with the effects mediated largely by the type of fatty acids that may be produced.

Faecal culture surveys performed during feedlot assembly of export sheep have documented an increase in Salmonella shedding during the assembly period, indicating that sheep become infected with Salmonella during the assembly period. The proportion of sheep shedding Salmonella prior to load out from the assembly depots ranges from 7 – 93%.[3, 13, 14] The Salmonella serotypes isolated from assembly yards, those shed by sheep during the assembly period, and those isolated from the tissues of sheep that have died of salmonellosis are all similar. [3, 13] When combined with the very low prevalence of Salmonella shedding (<1%) in sheep as they arrive at the assembly yards, these findings suggest that the assembly facility is the most likely source of Salmonella infection for many sheep that subsequently die of salmonellosis.[3, 13] Salmonella shedding exponentially amplifies Salmonella contamination of the environment. Infected animals may excrete 10^8 to 10^{10} Salmonella

per gram of faeces.[35] As environmental Salmonella contamination increases, the balance between challenge dose and herd immunity is tipped in favour of the pathogen. High sheep throughput has previously been proposed as a risk factor for salmonellosis during the assembly period.[13] As sheep are run through the same yards for receival and load out, high throughput will increase the Salmonella challenge encountered by sheep on arrival at assembly depots and when sheep are housed or handled in assembly paddocks or yards that have been used in previous assembly periods.

The virulence of Salmonella is variable between and within Salmonella serovars. Some serovars contain a virulence plasmid and are capable of causing disease in relatively healthy animals. Often Salmonella behaves as an opportunistic pathogen causing disease in the immunologically naïve or compromised host. The manifestations of salmonellosis in the live sheep trade are variable. Disease may be observed in compromised sheep that are nutritionally stressed having refused to eat the pelleted ration. Outbreaks of clinical salmonellosis were also observed in assembly depots affecting numerous lines of sheep. Dramatic examples of the impact of sheep source were observed on ship during the B.LIV.0123 investigation where high mortality in pens of sheep was traced to a specific line of sheep in the pen. Interestingly at the start of these outbreaks postmortems revealed the sheep had full rumens indicating that they had been actively consuming feed. These examples indicate that for some lines of susceptible sheep there are factors other than appetite contributing to their susceptibility to salmonellosis.

Experimental Salmonella challenge trials provide an insight into the course of the disease. Following challenge animals become febrile and anorexic within 36 – 72 hours. The onset of disease is more rapid when the challenge dose is large, the host compromised or the virulence of the infecting strain is high. Diarrhoea is usually observed within 24 hours of the onset of fever. When an animal is initially challenged the body mounts a response to eliminate the challenge. Salmonella have the capacity to invade cells and evade the immune response. If the host's immune response is rapid and effective the infecting strain is eliminated and clinical disease avoided. If the balance is in favour of the pathogen it will proceed to multiply until the host succumbs to the infection. When the challenge dose is large and or the virulence of the infecting strain is high, animals may succumb to infection within 48 hours. In most challenge experiments the majority of mortalities are observed between 3 to 10 days after challenge. Animals that survive 14 days following challenge are unlikely to die. In the live sheep trade the timing of Salmonella exposure will vary within the population according to stock management and environmental conditions. The same factors also influence the magnitude of the exposure. The suggestion by Richards et.al. (1989) that salmonellosis is responsible for more deaths early in the export process is consistent with this description of the pathogenesis of the disease. There are occasionally exceptions to this scenario. During the B.LIV.0123 investigation there were two high mortality lines that were observed to travel well for the first 9 – 10 days of the voyage and that subsequently experienced outbreaks of salmonellosis. In each of these cases conditions during the voyage were such that the pens that the sheep were in became wet preceding the outbreak. It is possible that the increased moisture in the pens favoured proliferation of the organism and subsequently increased the Salmonella challenge. What was particularly notable was that mortality was most notable in specific lines of sheep in the pens perhaps reflecting the contribution of other factors associated with increased susceptibility or lower immunity in these lines.

4.1.4 Salmonella vaccines

4.1.4.1 Introduction

Resistance to Salmonella infection involves innate and acquired immunity. Innate immune mechanisms provide the initial defence against infection in the naïve host and are not specifically directed against Salmonella. Acquired immunity reflects immunological memory and is stimulated by natural Salmonella exposure or Salmonella vaccination. In the live sheep trade, sheep are commonly exposed to Salmonella but most do not develop disease suggesting the innate resistance of most animals provides effective defence against Salmonella infection. Significant differences are observed between different lines of sheep with 75% of the total mortality observed in 16% of lines. [3]

Numerous experimental Salmonella vaccines have been developed over the years and these can be broadly divided into three categories: bacterin, subunit, and attenuated (modified live) vaccine. Salmonella bacterin and attenuated Salmonella vaccines are the only Salmonella vaccines available in Australia and New Zealand. A multi-strain, inactivated vaccine (Salvexin+B) is registered in New Zealand for use in sheep and cattle[a]. There are no Salmonella vaccines currently registered for use in sheep in Australia though Intervet/Schering-Plough Animal Health[b] currently produces a Salmonella Dublin / Salmonella Typhimurium Salmonella bacterin vaccine that is licensed for use in cattle. This vaccine is produced at their manufacturing facility in Bendigo. The Victorian Department of Primary Industry also has a GLP approved vaccine production facility which is used for the manufacture of custom autogenous Salmonella bacterins on an as needed basis. The autogenous vaccines are not registered for general sale. Bioproperties Pty Ltd, produce an aro attenuated Salmonella Typhimurium live vaccine[c], and this vaccine is registered for use in poultry.

The logistics of the live sheep trade are important to consider when considering the possible application of Salmonella vaccines. Vaccine delivery, safety, and efficacy are important factors that impact the cost and viability of immunoprophylaxis.

4.1.4.2 Vaccine delivery

In regards to the live sheep trade a Salmonella vaccine could be delivered on property of origin or on arrival at assembly depots.

a. On farm – Sheep are often purchased within three weeks of assembly. When sheep are scarce it is not uncommon for sheep to be purchased during the week preceding assembly. The implications for on farm vaccination are that the interval from vaccination to assembly is likely to be inconsistent and potentially shorter than desired. Vaccines that require multiple doses and/or that take weeks to stimulate immunity are unlikely to be effective. Vaccination of individual animals by injections performed on the farm may also pose significant vaccine packaging and delivery cost.

b. During assembly - Sheep husbandry practices at assembly depots around Australia have developed over the last 40 years to provide an efficient system for receiving and drafting large numbers of sheep in a short period of time. The emphasis of this process is to identify

[a] http://www.intervet.co.nz/binaries/90_169507.pdf
[b] http://www.intervet.com.au/
[c] http://www.bioproperties.com.au/vaccines/VaxsafeST.htm

sheep that are not fit for export, sort animals into like groups and to get all animals through this process rapidly and back on feed with minimal stress. Options for vaccinating sheep at assembly depots include:

i. Oral vaccine delivery via drinking water – This could be incorporated into current management practices with some capital expenditure to install vaccine delivery systems for water troughs (such as the Select Doser, marketed by Think Livestock[d]). This approach would reduce the cost of vaccination compared to options involving injection of individual animals as there would be minimal expenditure on packaging and delivery.

ii. Individual vaccination of sheep via intramuscular or subcutaneous injection – This approach is likely to be detrimental due the additional stress it would place on sheep and due to the increased Salmonella challenge that would be incurred through increased yarding and handling to allow vaccination. This approach could exacerbate rather than reduce risk and would also be associated with increased labour and product costs.

4.1.4.3 Vaccine safety

No vaccine is completely innocuous. In regards to Salmonella vaccines, killed products or bacterins have been associated with infrequent sporadic cases of anaphylactoid type reactions. Bacterins should not be administered during very hot weather or to stressed animals. The protection afforded by attenuated live Salmonella vaccines is achieved by the vaccine strain infecting the host and stimulating innate and acquired immune mechanisms. The margin of safety for different attenuated Salmonella vaccines varies according to the method of attenuation. Australian Pesticides and Veterinary Medicines Authority (APVMA) registration requires a minimum 10-fold margin of safety. Attenuated Salmonella vaccines infect and colonise the host and are shed in faeces for a few days following vaccination. Environmental contamination is likely and therefore there is potential for proliferation of the vaccine strain in the environment. The number of Salmonella present in the environment following proliferation does not normally exceed 10^7 organisms per gram. In the context of safety it would be important to evaluate the environmental behaviour of the vaccine and check that the subsequent potential exposure is within the recommended safety margin. Oral vaccination of sheep with 10^7 DNA Adenine Methylase (DAM) attenuated Salmonella does not adversely impact appetite or cause fevers (House, Unpublished data). Similar observations have been reported for aromatic dependent Salmonella Typhimurium vaccines in sheep.[36] Transient fevers and potentially some depression of appetite are not uncommon following vaccination with commercial vaccines. These responses are considered acceptable in normal production systems. For example the following quote is included in the precautions listed on the label for the Salvexin® +B Salmonella vaccine that is commercially available for sheep in New Zealand "Occasionally, for up to a week after vaccination, some ewes may develop symptoms ranging from mild loss of appetite through to ataxia, recumbency and death[e]." What may be acceptable on a farm may not be acceptable for sheep entering an assembly depot where additional stressors have the potential to have adverse implications on getting sheep settled and back on feed.

[d] http://www.thinklivestock.com
[e] http://www.spah.co.nz/dyn_documents/t_product_documents-label_salvexin_b.pdf

4.1.4.4 Salmonella bacterins

There are currently no Salmonella bacterins licensed for use in sheep in Australia. The only Salmonella bacterin licensed for use in Australia is Bovilis® S a *S. enterica* serovar Typhimurium / *S. enterica* serovar Dublin bacterin registered for use in cattle. The product label recommends that cows should be vaccinated twice with a 2 ml subcutaneous injection 3 to 4 weeks apart, followed by an annual booster. For colostral protection, it is recommended that pregnant cows be vaccinated approximately 8 and 3 weeks before calving[f].

A multivalent *S. enterica* serovar Typhimurium, *S. enterica* serovar Bovismorbificans, *S. enterica* serovar Hindmarsh, *S. enterica* serovar Brandenburg vaccine for sheep is sold in New Zealand. The recommended application of this vaccine involves administering two doses of the vaccine 3 – 4 weeks apart with the second dose administered 3 weeks prior to the anticipated Salmonella exposure. These vaccines have been reported to reduce the incidence and severity of disease and mortality in flocks infected with Salmonella Brandenburg.[37] However they were not observed to provide significant protection against mortality or abortion in experimental challenge studies.[19]

There are conflicting reports regarding the efficacy of Salmonella bacterins with some reports suggesting they are not effective and others indicating they are capable of stimulating partial protection. The overall consensus of Salmonella bacterin trials across species suggest these vaccines provide partial protection against Salmonella challenge.[38-43]

Adverse anaphylactic reactions are occasionally reported after administration of Salmonella bacterins. The cause of these reactions is unknown but has been suggested that it is associated with the lipopolysaccharide content of these products.[44-49]

Characteristics of Salmonella bacterins that limit their application in the live sheep trade include the likely requirement for multiple doses, the associated time required to induce immunity, and the possibility of limited efficacy in protecting against morbidity and mortality.

4.1.4.5 Attenuated (modified live) Salmonella vaccines

Comparative vaccine trials indicate attenuated Salmonella vaccines provide greater protection against virulent Salmonella challenge than Salmonella bacterins.[50-53] Vaccination with modified live Salmonella vaccines attenuates the severity of clinical signs, reduces Salmonella shedding and reduces mortality.[54-56]

4.1.4.5.1 Aro-attenuated Salmonella vaccines

A number of naturally occurring and genetically manipulated attenuated Salmonella strains have been used to vaccinate poultry and livestock against salmonellosis. Of these the only live attenuated Salmonella vaccine currently registered for use in Australia is an aromatic dependent *S. enterica* serovar Typhimurium vaccine produced by Bioproperties Pty Ltd. Aromatic amino acid (aro) and purine (pur) auxotrophs of Salmonella are attenuated and have decreased virulence.[57-61] Numerous trials with aromatic auxotrophs have been conducted in sheep.[22, 23, 59, 62-65] In these trials vaccine delivery via intramuscular injection provided more robust protection than oral vaccination.[59]

[f] http://www.intervet.com.au/binaries/82_103325.pdf

Sheep vaccinated with aromatic dependent *S. enterica* serovar Typhimurium vaccine were protected when the challenge was conducted 3 weeks following vaccination.[21] Oral vaccination did not afford protection when sheep were challenged 7 and 14 days following oral vaccination.[64] The protection afforded by aromatic attenuated Salmonella vaccines is serotype specific 3 weeks following vaccination[66] requiring the development of a multivalent vaccine containing the appropriate disease causing serotypes. To this end *S. enterica* serovar Typhimurium, *S. enterica* serovar Bovismorbificans, and *S. enterica* serovar Havana aro mutants have previously been developed.[23, 63, 64]

Registration of the aromatic *S. enterica* serovar Typhimurium vaccine has previously been investigated. Limitations with this approach included the necessity for sheep to be vaccinated on the property of origin 3 weeks prior to arrival at the assembly depot. Ideally an aromatic vaccine administered at this time would also include multiple Salmonella serotypes to provide protection against the relevant Salmonella serotypes. Inclusion of multiple serotypes increases the cost of production as there is a cost incurred for each serotype included in the vaccine. Aromatic attenuated Salmonella vaccines also induce transient immunosuppression having the potential to exacerbate an existing Salmonella infection.

4.1.4.5.2 DNA Adenine methylase (DAM) attenuated Salmonella vaccines

DNA methylation plays a role in regulating the expression of DNA. When the DNA Adenine Methylase gene is removed the resulting organism is unable to regulate DNA expression normally and is attenuated. Features of this attenuation which have proved exciting from the perspective of vaccination include the capacity of DAM Salmonella vaccines to induce homologous and heterologous protection, the capacity of these vaccines to upregulate innate immune mechanisms and the absence of the immunosuppression observed with other attenuated Salmonella vaccines. [67-74]

Of particular relevance to the live export industry is the capacity of DAM attenuated Salmonella vaccines to protect against homologous and heterologous challenge and the capacity of DAM attenuated vaccines to enhance innate immunity within 24 hours of vaccination. Early vaccine induced resistance may be mediated by competitive exclusion between the vaccine strain and virulent organisms,[75] and or by induction of polymorphonuclear cells in the lamina propria of the small intestines.[76] Innate immune mechanisms are also up-regulated by non-specific activation of macrophage functions with the involvement of TNF alpha, IL12, IFN gamma, and NK cells.[77-79] Longer-term protection requires antigen-specific recall of immunity involving the involvement of B and T cells.

Experimental challenge trials with DAM vaccines have been conducted in mice, poultry, and cattle.[68, 70-73, 80, 81] These trials have produced similar results demonstrating the capacity for induction of homologous and heterologous protection in each species. Recently one of the authors (J House) has been involved in conducting preliminary trials in sheep. Previous studies in poultry and cattle have focused on oral vaccination of neonates. A potential limitation in adults is interference from the rumen flora following oral vaccination of adult livestock. Oral delivery of the vaccine with a dose of 10^7 was found to be effective at establishing the vaccine in adult sheep and was not associated with any adverse vaccine reactions or anorexia. The performance of this vaccine in other species suggests it may be well suited to application in the live sheep trade as it provides homologous and

heterologous protection and could be delivered in drinking water. However, further trials in sheep would be required for APVMA registration.

The efficacy of DAM attenuated Salmonella vaccines have been evaluated in mice, chickens, and calves. In each species the vaccine was found to provide protection against virulent challenge. DAM Salmonella vaccines have proved protective against homologous and heterologous Salmonella challenge where the experimental sample size has required a minimum 40% improvement in outcome to achieve a statistically significant outcome.[68, 70-73, 80, 81] Salmonella species are grouped according to their outer membrane antigenic properties into serogroups. The serogroups most frequently implicated in disease in livestock belong to serogroups B, C, D, and E. Heterologous studies have been conducted with DAM attenuated Salmonella vaccines using a serogroup B derived vaccine challenged with Salmonella from serogroups B, C, D, and E. The greatest protection afforded has been observed to homologous Salmonella challenge. However, the protection to diverse heterologous Salmonella challenge has also been significant. Experiments evaluating the efficacy of DAM attenuated Salmonella vaccines have included traditional vaccination trials that have assessed induction of acquired immunity and an experiment in calves that evaluated induction of rapid non-specific immunity, within 24 hours of vaccination.[71] In the latter study which is of relevance to assembly delivery of vaccine in the live export industry, 11 of 18 vaccinated and 4 of 19 non-vaccinated calves survived virulent Salmonella challenge 24 hours following vaccination. This represents a 58% reduction in mortality. Neonatal salmonellosis is a common disease problem in dairy calves and maternal vaccination provides limited protection to calves which typically get sick and die between 10 – 28 days of age. The logic of the study was to determine if this live vaccine could up regulate innate immune mechanisms in an immunologically vulnerable host to protect against Salmonella challenge prior to induction of adaptive immune mechanisms.

When sheep enter an assembly depot there is exposure to Salmonella although the timing of Salmonella challenge will vary between animals in the population. The interval between vaccination and Salmonella challenge will subsequently be variable. Optimal protection would be achieved when this interval is greater than 2 weeks. The logistics of delivering vaccines to sheep on farm prior to assembly are challenging considering that it is not uncommon for sheep to be purchased during the week prior to assembly. On-farm vaccine delivery also significantly increases the cost of vaccination due to the need for individual animal delivery requiring increased packaging, dispensing equipment, and labour.

From an immunological perspective vaccine delivery via drinking water on arrival at assembly depots does reflect a compromise. Animals may be exposed to Salmonella on arrival (before they are vaccinated) or within a relatively short interval after oral vaccination. Although higher levels of environmental contamination and exposure may not occur until later in the assembly period. However, this approach also offers a more affordable, practical, minimal stress vaccine delivery option. Considering that 99% of sheep survive the live export process it suggests that for the most part the magnitude of Salmonella challenge is not high. This is in contrast to the situation in most experimental challenge studies where animals are challenged with 10^{10} to 10^{11} virulent organisms. Given that a 40% reduction in mortality was observed in calves challenged with a large dose of Salmonella 24 hours following vaccination with the orally delivered DAM vaccine a greater reduction in mortality would be expected when the challenge dose is low or where the interval from vaccination to challenge is longer than 24 hours.

4.1.4.5.3 Other live attenuated Salmonella vaccines

There are numerous other experimental live attenuated Salmonella vaccines that have been investigated in different species. These include rough mutants, temperature sensitive mutants, and gene deleted strains. Genes that have been targeted for the construction of vaccines include those involved in the biosynthesis of bacterial lipopolysaccharide (galE), regulation of expression of outer membrane proteins (ompR), amino acid or purine biosynthesis (e.g. aro, pur, guaB), regulation of carbon source utilization (cya crp), virulence factors and others, such as htrA, phoPQ, recA and waaN.[82] Few mutants have been tested in sheep and there are no reports of these vaccines inducing the cross protection that has been observed with DAM vaccines.

4.1.4.6 Subunit Salmonella vaccines

Outer membrane proteins and ribosomal extracts have been applied in Salmonella vaccines. Most of this work has been done in mice and poultry.[83, 84] Experiments in calves demonstrated limited protection.[85] There are no reports describing the use of Salmonella subunit vaccines in sheep.

4.1.4.7 DAM attenuated Salmonella vaccine intellectual property

The efficacy of DAM attenuated Salmonella vaccines was initially discovered by Professor Michael Mahan at the University of California, Santa Barbara. The University of California currently holds the patents for these vaccines in the United States. The patents held by the University of California do not cover the use of these vaccines in Australia so there would be no associated license fee.

University of California US patents include: 6,365,401; 6,548,246; 7,026,155. These can be viewed by typing in the patent no. at the USPTO website http://patft.uspto.gov/netahtml/PTO/srchnum.htm

4.1.4.7.1 DAM attenuated Salmonella vaccine royalties

The DAM vaccine experiments that have been conducted in poultry, cattle, mice, and sheep have utilised the UK1 *S. enterica* serovar Typhimurium strain that was attenuated by Professor Mahan's group. If this vaccine strain were to be utilised as a vaccine by the live sheep trade it would be subject to royalties payable to Professor Mahan. The alternative would be to delete the DNA Adenine Methylase gene from an Australian isolate. There is a good track record with the UK1 *S. enterica* serovar Typhimurium strain and reports with other Salmonella vaccines would suggest that the effectiveness of different parent strains is variable. Engineering a new strain is likely to work but may require more than one attempt as well as attendant costs associated with efficacy testing. As the University of California patent does not apply to Australia it should be possible to patent the vaccine in Australia.

One of the authors (J House) has been and continues to be involved as a collaborator in research studies associated with efficacy and safety of DAM attenuated Salmonella vaccines in livestock. Discussions have been held with Professor Mahan concerning the issue of royalties and the authors are confident that an agreement can be reached which is practical and reasonable.

4.1.4.7.2 Current status of DAM Salmonella vaccines in sheep

Preliminary unpublished vaccination studies have been conducted in sheep to evaluate safety and the appropriate vaccine dose (J House). Preliminary studies have also been conducted to evaluate the stability of the vaccine in water and the propensity of the vaccine to proliferate in the environment. Experimental vaccine efficacy studies are scheduled to commence later this year, these studies are part of ongoing research that is been funded by the United States Department of Agriculture to evaluate the efficacy of these vaccines in adult ruminants. Sheep provide a more practical and cost effective model than cattle for these types of experiments. It would be logical to wait for the results of these trials prior to pursing registration of the DAM vaccine for use in the live sheep trade. Prior to pursuing vaccine registration it would also be logical to conduct field trials to assess the efficacy of the vaccine in the live sheep trade. These field trials should be conducted in accordance with the requirements of the registration process so that the results could be submitted as part of the registration package.

4.1.5 The vaccine registration process

Registration of veterinary vaccines in Australia is managed by the APVMA with the process defined in Guideline 47 “Data requirements and guidelines for registration of new veterinary immunobiological products”. AQIS approval would be required prior to registration of the California derived DAM Salmonella vaccine, this would not be required if a local Salmonella isolate were engineered as the parent vaccine strain. All genetically modified vaccines also require approval by the Office of the Gene Technology Regulator.

Veterinary vaccine registration requirements include:

1. The vaccine must be manufactured to a standard comparable with the Australian Code of Good Manufacturing Practice for Veterinary preparations

1. The formulation of the vaccine has to be clearly defined including all active constituent(s): maximum and minimum release titres and end of shelf-life titre.

2. The specifications for the immediate container and stoppers/closures (including acceptable tolerances) must be supplied

3. A flow chart of the manufacturing process must be provided, showing each step from production of the active constituent to formulation of the final product in final containers, including any critical in-process control testing steps.

4. Raw materials used in the manufacture of the vaccine have to be defined

5. Genetically modified organisms have to be approved by the Office of Gene Technology Regulator and tests conducted to verify stability and the potential for reversion to virulence.

6. Details need to be provided regarding the master and working seed, media preparation, and for control tests conducted during production and on the final product.

7. Stability of the vaccine has to be established to determine the shelf life.

8. Meat withholding periods need to be established for potentially zoonotic vaccines.

9. The implications of the vaccine on Australian trade have to be addressed.

10. The implications for occupational health and safety have to be addressed.

11. The potential for environmental exposure have to be addressed.

12. Efficacy and safety data have to be supplied, this is derived from a combination of pen and field trials. Live vaccines need to have a minimum 10 fold safety margin.

Currently AQIS and the APVMA have a backlog of dossiers to approve. The time required for registration is therefore influenced by the time taken to produce and collate the dossier and the time taken for processing of the dossier by government regulatory agencies.

4.2 Economic analysis

4.2.1 Model description

The analysis builds on that undertaken by Clarke (2004).[86] It purposefully uses similar methodology and where possible similar assumptions in order to facilitate comparison of results. Vaccine is applied through water supply systems at the assembly point, therefore all costs, management and levels of production incurred before this point are the same for the 'with' and the 'without' vaccination scenarios. The starting point for the analysis is the cost of sheep delivered to the feedlot. The value of the sheep at this point reflects all on-farm costs and transport costs to the feedlot. The analysis then compares, using discounted Benefit/Cost Analysis (BCA), the 'without vaccination' scenario where Salmonella continues to cause losses both at the feedlot and on the boat with the 'with vaccination' scenario where losses are reduced. Sensitivity analysis is undertaken with regard to the important variables that may affect the economic viability of an industry wide vaccination program. These variables are:

- Sheep exports
- Mortality rates
- Sheep prices
- Vaccine and vaccination costs

Table 1 provides the input values used for those variables in the model that were assessed over a range of values. The 'negative' column reflects the level of parameters that will have a negative effect on the Benefit/Cost ratio (BCR). Negative and positive values were calculated by varying 25% either side of the expected values.

Table 1: Parameter estimates used to identify those inputs that affect program viability. Negative, expected and positive values were used to assess the impact of inputs under a range of scenarios.

	unit	Negative	Expected	Positive
Sheep exported from Fremantle	'000 hd	2025	2700	3375
Sheep exported from Portland	'000 hd	390.75	521	651.25
Sheep exported from Adelaide	'000 hd	368.25	491	613.75
Mortality rate in feedlot - typical	%	0.08375	0.067	0.05025
Mortality rate in feedlot - spike	%	1.25	1.00	0.75
Consignments with Salmonella spikes	%	20	5	0
Consignments wih Salmonella spikes (2)	%	6.25	5	3.75
Sheep culled compared to spike mortalities	%	62.5	50.0	37.5
Mortality rate on boat attributable to Salmonella	%	0.3375	0.27	0.2025
Reduction in mortality rate due to vaccination	%	30	40	50
Reduction in rate of spikes due to vaccination	%	18.75	25	31.25
Exchange rate	US$	1	0.82	0.62
Sale price; delivered to destination	US$	79	105	131
Vaccine cost	$/dose	0.15	0.12	0.09
Vaccine registration cost	$'000	625	500	375
Total vaccination equipment costs at feedlots	$'000	625	500	375

4.2.1.1 Sheep exports

The analysis uses total sheep numbers exported from the three major live sheep export ports. In 2007 there were 2,700,000 exported from Fremantle (Western Australia), 521,000 from Adelaide (South Australia) and 491,000 exported from Portland (Victoria). [2] The analysis does not differentiate between different types and ages of sheep. The analysis assumes that all exported sheep are vaccinated at the feedlot. Even though there may be seasonal and locality issues that affect the incidence of outbreaks of the bacteria, these are not included in the analysis. It is expected that that the larger the number vaccinated the greater the economic efficiency of the program and the greater the likelihood that vaccine production and distribution will be economically viable.

4.2.1.2 Mortality and cull rates

Sheep mortalities occur either at the feedlot or on the boat. Mortality rate assumptions employed by Clarke (2004) were based on a an expected mortality rate of 0.067% in 80% of assembly feedlot periods and mortality rate spikes of up to 1% in the remaining 20% of assembly periods.[86] Observations from the more recent LIVE.123 project reported very similar mortality rates in assembly feedlots (0.06%) and as a result the same expected mortality rate of 0.067% was used in the current report as had been used by Clarke (2004). Spikes or feedlot periods with increased mortality up to 1% were observed less frequently and were estimated to occur in as many as 5% of assembly periods (J. House, Unpublished data). The current report therefore uses an expected value of 5% for the percentage of consignments that incur a spike in mortality due to salmonellosis. This parameter was then varied from 0% to 20% to assess the effect of considerable variation in salmonellosis.

When a salmonellosis event occurs (spike in mortality attributed to salmonellosis), there is an associated impact on in-contact sheep, particularly those animals in the same lines or assembly paddocks/yards as salmonellosis-affected sheep that do not succumb to salmonellosis or that do not get infected at all. Clarke (2004) states that 'once 30 cases of Salmonella are observed in any one 1,200 head feedlot paddock, the whole paddock must be 'pulled' from the trade'.[86] Clarke (2004) then estimated that the annual impact of mortality spikes (in addition to losses directly due to deaths) was that an additional 4,800 sheep were culled at salvage prices.[86]

Vaccination was assumed to have effects on both the mortality rate due to salmonellosis and on the incidence of mortality spikes. Vaccination was assumed to reduce mortality rate by 40%, meaning that mortality rate with vaccination was equal to the mortality rate in unvaccinated sheep multiplied by 1-0.4 or 0.6. This adjustment was applied to both the 0.067% and 1% mortality rates that applied to usual and spike mortalities, respectively. In addition, vaccination may be expected to reduce the incidence of spikes of mortality meaning that in a higher proportion of occasions the background or usual mortality would apply. It was assumed that vaccination would reduce the occurrence of spikes of mortality by 25%, meaning that the expected value for percentage of consignments that incur a spike in mortality due to salmonellosis is estimated as 0.75*0.05 = 0.0375 or 3.75%.

It is difficult to accurately estimate the number of animals lost due to salvage sale or carry-over of in-contact sheep. It is recognised that these losses will be influenced directly by the extent and severity of mortality eg number of paddocks or yards affected with salmonellosis and mortality rates. Losses due to salvage sale of animals direct to slaughter are likely to exceed losses associated with additional feed costs for sheep that are carried over to a later shipment.

The current report has implemented a simple and direct relationship between the number of sheep that die during a salmonellosis spike and associated losses due to salvage sale and carry-over feeding. A single figure called ***culling loss*** is used to estimate the total number of sheep lost due to both salvage sale and carry-over feeding and is calculated by assuming that the total number of sheep culled is 50% of the total number that die during spikes of Salmonella within the feedlot.

In eastern feedlots observations made during B.LIV.0123 suggested that all culled animals were sold for salvage value while in western feedlots almost all animals appeared to be carried over and fed in paddocks and then incorporated into a later shipment. As a result the model was parameterised to separately estimate and deal with cull animals in eastern and western feedlots. This was done by incorporating a parameter that defined the percentage of culled animals that were sold for salvage slaughter. The remaining percentage of culled animals were then retained and carried over to the next shipment. In eastern feedlots all animals were assumed to be sold for salvage at a value of $20 per head. In western feedlots all animals were assumed to be held over and fed for an additional two weeks at the standard daily rate assigned to all feedlot animals.

4.2.1.3 Sheep prices

The three prices used in this analysis are the purchase price (value delivered to the assembly feedlot), the cull sale price and the sale price for live sheep landed in the Middle East (Cost Insurance Freight, CIF). While it is possible that sheep may be sold at the port of loading (i.e. Free on Board, FOB) this transaction is not included here. The inclusion of this extra point of sale has no impact on the analysis. The expected price for sheep landed at the assembly feedlot is $60 per

head, this includes purchase price from the farm and transport to the feedlot. While it is an important variable with regard to profitability it has no impact on the viability of the vaccination program as the same cost is applicable to both the 'with' and 'without' vaccination scenarios.

Salvage price for cull sheep sent from assembly depot to slaughter was assumed to be $20 per head. Cull sheep prices may vary significantly depending on season and market demand.

Sale prices for sheep in the Middle East were not expected to be highly variable, but the fact that contracts are written in US dollars ensures that this variable will be important.

4.2.1.4 Vaccine and vaccination program costs

The number of facilities that can produce vaccines in Australia are limited. Intervet/Schering-Plough Animal Health have a facility in Bendigo, the Department of Primary industries in Victoria have a facility and Bioproperties Pty Ltd has a GMP facility in Glenorie NSW.

Tony Fahy from the Department of Primary Industries in Victoria provided the estimated production costs of 12 to 17 cents per dose. The cost per dose is largely influenced by the number of doses produced. The cost will also be influenced by the royalty payments required for use of the vaccine strain. The model assumes that a royalty payment is incorporated in the 12 cent expected value of the unit cost of vaccine production. There is no licensing fee associated with the intellectual property of the vaccine. The previous estimate[86] for registration cost was approximately $500,000 and following review of the requirements, this value is once again used as the expected cost for registration.

There will also be equipment needed to be purchased by feedlots to administer the vaccine. This would entail the purchase of approximately 20 units of equipment that can ensure accurate dosage of the vaccine into the feedlot water supply. Sheep would only need access to the vaccine-impregnated water during their time in feedlot. It is expected that there are about 6 or 7 assembly points that would need to access this equipment. It is estimated that the total costs to the industry would be approximately $500,000. The costs of registration will be incurred in Year 1 while the cost of equipment is incurred in year 3 when the vaccination program begins.

4.2.1.5 Other costs

There are additional costs identified that do not vary during the analysis, they are not regarded as being linked to the Salmonella vaccination program. These costs are provided in Table 2.

Table 2: Description of expected values for other parameters that were not varied in economic modelling

		unit	Expected value
Sheep purchase price; delivered to the feedlot		$/hd	60
Sale price for cull sheep (salvage to slaughter)		$/hd	20
% of cull sheep that are sold (remainder carried over to next shipment)			
	East coast	%	100%
	West coast	%	0%
Transport			
	From feedlot to ship	$/hd	5
	Portland to Middle East	$/hd	35
	Adelaide to Middle East	$/hd	35
	Fremantle to Middle East	$/hd	30
Other feedlot costs			
	Feed costs at East Coast feedlots	$/hd	3
	Handling costs at East Coast feedlots	$/hd	2.3
	Feed and handling costs at West Coast feedlots	$/hd	7
	AQIS	$/hd	0.12
	Veterinarian	$/hd	0.1
Other shipping costs			
	Insurance	$/hd	0.4
	Finance	$/hd	0.75
Discount rate		%	5
Vaccine program begins		year	3

4.2.2 Results

Using the parameters identified in Tables 1 and 2, and with all input parameters set at expected values, the vaccination program will be economically viable. Results of expected value analyses, sensitivity analyses and break-even analyses are presented in Tables 3 and 4.

The export industry is estimated to be generating gross income in excess of $473 million per annum. In this context a vaccination program with start-up costs of $1m and annual costs of $445,000 is not difficult to handle in an economic sense at an industry level.

The vaccination program is expected to have a discounted benefit (Net Present Value or NPV) of $507000 to the industry after 10 years with a BCR of 1:1.13 (using a discount rate of 5%). This implies that for every dollar invested there will be a benefit to the industry of $1.13. If money was borrowed by the industry to cover startup and annual costs, the interest rate would need to increase to 17% (Internal Rate of Return or IRR) before the program would become unviable.

The benefits are minor when considered on a return per sheep basis. When the exchange rate is set at 0.82, the gross margin per sheep without the vaccination program is $16.79, while for the vaccinated sheep an increase of only $0.06 per sheep to $16.85.

The first row in Table 3 shows results when all input parameters are held at expected values. Subsequent rows show model output when one variable at a time is changed and all other variables are held at expected values. Model output is expressed as net present value (NPV), benefit-cost ratio (BCR) and internal rate of return (IRR), each calculated after 5 and 10 years. Table 4 provides breakeven values for vaccine program costs and benefits represented by values for parameters where the estimated NPV = 0.

Table 3: Results from economic analyses. The first row shows results when all input parameters are held at expected values. Subsequent rows show model output when one variable at a time is changed and all other variables are held at expected values. Model output is expressed as net present value (NPV), benefit-cost ratio (BCR) and internal rate of return (IRR), each calculated after 5 and 10 years. NA= not able to be estimated

	Input values	NPV ($,000)		BCR		IRR	
		5 yrs	10 yrs	5 yrs	10 yrs	5 yrs	10 yrs
Expected values for all variables		**-312**	**507**	**0.86**	**1.13**	**-15%**	**17%**
Changed variable (expected value)	**Changed input**						
(all other variables held at expected values)							
Sheep exported from Fremantle (2,700,000)	2,025,000	-418	255	0.79	1.07	-23%	11%
	3,375,000	-205	760	0.92	1.16	-8%	22%
Sheep exported from Portland (521,000)	390,750	-334	455	0.85	1.12	-17%	16%
	651,250	-290	560	0.87	1.13	-13%	18%
Sheep exported from Adelaide (491,000)	368,250	-332	458	0.85	1.12	-17%	16%
	613,750	-291	557	0.87	1.13	-14%	18%
Feedlot mortality rate (0.067%)	0.08375	-264	621	0.88	1.15	-12%	19%
	0.05025	-359	394	0.84	1.10	-19%	15%
Spike mortality rate (1%)	1.25	-252	648	0.89	1.16	-11%	20%
	0.75	-371	366	0.83	1.09	-20%	14%
Consignments with spikes (5%)	20	360	2,101	1.14	1.43	24%	46%
	6.25	-256	640	0.89	1.16	-11%	20%
	3.75	-368	375	0.83	1.09	-19%	14%
	0	-535	-23	0.75	0.99	NA	4%
Culling loss (% of mortalities: 50%)	100	-285	572	0.87	1.14	-13%	18%
	62.5	-305	523	0.86	1.13	-15%	17%
	37.5	-319	491	0.86	1.12	-16%	17%
Salmonella mortalities on boat (0.27%)	0.3375	5	1,258	1.00	1.31	5%	32%
	0.2025	-628	-244	0.72	0.94	NA	-2%
Reduction in mortalities due to vacc (40%)	30	-711	-439	0.68	0.89	NA	-9%

	50	87	1,454	1.04	1.35	10%	35%
Reduction in spikes due to vacc (25%)	18.75	-327	471	0.85	1.12	-16%	16%
	31.25	-296	544	0.87	1.13	-14%	18%
Exchange rate (0.82 USD = 1 AUD)	1	-653	-302	0.71	0.93	NA	-4%
	0.62	299	1,957	1.13	1.48	21%	43%
Sale price at destination ($105USD)	$79	-789	-624	0.65	0.85	NA	NA
	$131	165	1,639	1.07	1.41	14%	38%
Vaccine cost (0.12)	0.15	-587	-146	0.77	0.97	NA	1%
	0.09	-37	1,160	0.98	1.34	3%	30%
Vaccine reg cost ($500,000)	$625,000	-431	388	0.82	1.09	-19%	13%
	$375,000	-193	626	0.91	1.16	-10%	22%
Total vacc equipment cost ($500,000)	$625,000	-420	399	0.82	1.10	-21%	14%
	$375,000	-204	615	0.90	1.16	-8%	20%

Table 4: Breakeven values for vaccine program costs and benefits represented by values for parameters where the estimated NPV = 0.

Variable		Expected value	Breakeven value at 10 yrs	Breakeven value at 5 yrs
Mortality rate in feedlot - typical	%	0.067	NPV positive at 0%	0.177
Mortality rate in feedlot - spike	%	1.00	0.101	2.32
Consignments with Salmonella spikes	%	5	0.23	12.0
Sheep culled compared to spike mortalities	%	50.0	NPV positive at 0%	625
Mortality rate on boat attributable to Salmonella	%	0.27	0.225	0.337
Total sheep exported	n	3,712,000	2,400,000	4,200,000
Reduction in mortality rate due to vaccination	%	40	34.65	47.85
Reduction in spikes due to vaccination	%	25	NPV positive at 0%	NPV negative at 100%
Exchange rate	US$	0.82	0.924	0.704
Sale price delivered to destination	US$	105	93.4	122
Vaccine cost	$/dose	0.12	0.143	0.086
Vaccine registration cost	$'000	500	1,025	170
Total vaccination equipment costs at feedlots	$'000	500	1,080	130

4.2.2.1 Sensitivity analyses

Sensitivity analyses were conducted to provide additional information on the impact of changing one input parameter at a time on model outputs. All other input variables were held at their expected values in this approach. While this approach is simplistic and does not take into account interrelationships between the input variables, it does provide valuable insight into the impact of changes in either direction of each input variable and the level of sensitivity of the model to specific variable input values.

Individual parameter values were varied by 25% of their expected value in either direction for sensitivity analyses (Table 1). The 'negative' column indicates that this change, whether it be an increase or decrease in its value, will have a negative effect on viability. Likewise, the 'positive' column provides the change in value that will improve viability.

In some cases additional parameter values were utilised for particular parameters of interest. Examples included estimates of the percentage of consignments that experience spikes of mortality due to salmonellosis. Clarke (2004) estimated that 20% of all consignments experienced spikes of mortality due to salmonellosis. Observations during LIVE.123 suggested that values of 5% were more representative of the last several years of shipments. The main parameters for this variable were therefore set at an expected value of 5% and a 25% variation either side of this value (3.75% to 6.25%). Additional values of 20% and 0% were added in sensitivity analyses to test for extreme values and to assess the parameter value as used by Clarke (2004).

Estimates of the number of sheep culled from assembly feedlots when a spike of salmonellosis occurred were difficult to parameterise. The expected value was linked to the number of mortalities due to salmonellosis and was estimated as 50% of the total deaths due to salmonellosis. This value was then varied by 25% in either direction. An additional value equal to 100% of the total mortalities due to salmonellosis was also added to the sensitivity analyses to test for more severe culling as a result of salmonellosis.

Break-even analyses were conducted by setting all input parameters at expected values and then changing one parameter value at a time until the NPV was either zero or positive but very close to zero (Table 4). This produced estimates of threshold values for input parameters that defined a border between viability and non-viability of vaccination in an economic sense. Comparison of the break-even values for 5-year and 10-year NPV estimates allow some assessment of the window or range of values for each parameter that may demarcate viability of a vaccination program.

The five most influential input parameters based on economic outputs and sensitivity analyses, were exchange rate, sale price at destination, reduction in mortalities due to vaccination, Salmonella mortalities on boat, and the unit price of the vaccine.

4.2.2.1.1 Mortality rates in feedlots

Model outputs in Table 3 changed relatively little in response to 25% variation from expected values for either typical mortality rate in feedlots or the spike mortality rate in feedlots, suggesting relative insensitivity to these input parameters. The expected values for these two parameters are numerically small and 25% variation is still a small change.

It is noteworthy that break-even analyses (Table 4) indicate that even when the usual or background mortality rate in feedlots due to salmonellosis is reduced to zero, the 10-year NPV estimate remains positive. In addition as long as spike mortality in feedlots is greater than or equal to 0.101% (a reduction of 90% from the expected value of 1%), the 10-year NPV remains positive indicating that vaccination still has a positive economic benefit.

In these situations the positive impact of vaccination is driven by impacts on mortalities on boat attributable to salmonellosis which has a higher expected value. This is reinforced by the 10-year breakeven value for mortality rate on boats (0.225), a reduction of 17% from the expected value.

The 5-year breakeven values for feedlot mortality estimates (typical and spike) are presented as an indication of the level of increase in severity of feedlot mortalities due to salmonellosis that would make a vaccination program breakeven within 5 years. While these values may be higher than expected they may also be considered to be biologically plausible. The impact of these sensitivity analyses suggests that even when feedlot mortalities attributed to salmonellosis are very low indeed, or if they increase slightly from expected values, vaccination programs are likely to be beneficial.

4.2.2.1.2 Salmonella spikes

The percentage of shipments that are affected with a spike of salmonellosis is set at an expected value of 5%. Variation of this value by 25% in either direction has relatively little impact on model outputs.

Breakeven analysis indicates that if the percentage of shipments with Salmonella spikes is 0.23% or higher (lower value is equivalent to 5% of the expected value of 5%), the model still indicates that vaccination has a positive NPV at 10 years. Retention of a positive NPV at 10 years even when there are very few spikes of mortality in the feedlot is being driven mainly by mortalities attributed to salmonellosis during the shipboard voyage component of the export process.

An increase in the % of shipments that incur salmonellosis spikes to 12% is sufficient to result in a positive NPV at 5 years.

The culling loss variable is an attempt to measure the impact of salmonellosis spikes in a feedlot that are due to forced culling of in-contact sheep from a particular shipment and reflects sheep that are either carried over in the feedlot to the next shipment (incurring additional feed and handling costs) or sheep that are sold to slaughter at a salvage price. Variation of the parameter value by 25% in either direction has very little impact on model outputs. Even if no sheep were culled at all in association with a spike of salmonellosis (input value = zero), the 10 year NPV remains positive. In addition, a 6.25 fold increase in culling would need to occur in order to make the 5 year NPV estimate positive, again reinforcing the fact that this variable plays relatively role in determining the economic value of vaccination against salmonellosis.

4.2.2.1.3 Mortality rate on boat

Mortality rate on boats due to salmonellosis is a more influential variable than the other health impact measures discussed above. Variation of the input value by 25% produces larger impacts on NPV estimates. The breakeven value for 10 year NPV of 0.225% provides an estimate of the threshold of viability. If the mortality rate on boats were to fall below this level, then vaccination would be unlikely to have a positive economic benefit.

The importance of this input parameter is also supported by the 5 year breakeven value for NPV of 0.337% which indicates that the level of increase in mortality rate on boats attributable to salmonellosis that would make the vaccination program produce a positive NPV within 5 years.

4.2.2.1.4 Reduction in mortality due to vaccination

The reduction in mortalities due to vaccination is one of the four most influential input parameters for the model along with the exchange rate, sale price at destination, and mortality rate on boat attributable to Salmonella. The breakeven value for the 10 year NPV is 34.65% indicating that if the mortality rate in vaccinated sheep is greater than 65.35% (100-34.65), vaccination is unlikely to be economically viable.

The importance of this input parameter is also reflected by the impact of change in the other direction. If the vaccine were to provide better protection than expected, then the economic analysis rapidly reflects a large positive effect on NPV and other model outputs. In fact if the reduction in mortalities due to vaccination was 47.85% or higher then the 5 year NPV estimates are positive (reflecting a mortality rate in vaccinated animals of 52.15% or below).

Evidence provided in the literature review component of this report supports the expected value for reduction in mortality due to vaccination (40%) that was used in this report. While there is some data to suggest that the impact may be better than this, it is acknowledged that there are limited data on the efficacy of the vaccine in sheep and that further studies are needed to assess and define this value.

4.2.2.1.5 Sale price and exchange rate

Sale price at the destination is the most influential input parameter in terms of the ability to adversely impact the 10 year NPV, while the exchange rate is the most influential in terms of ability to positively impact the 10 year NPV.

If the sale price declines to USD$93.4 or below, the program will not be viable under present conditions. All things being equal any change that leads to the final product being of higher value will improve the relative viability of the program. As the program provides more stock for sale in the Middle East an improvement in price will have a positive effect on the economic indicators.

Exchange rate has a similar effect and the impact of exchange rate variability is felt particularly through sale price though it is accepted that exchange rate may have minor impacts on other input costs including price of feed on the boat. As a general rule costs will increase as the Australian dollar gets weaker and the cost will decrease as the dollar gets stronger.

4.2.2.1.6 Total sheep exported

While outside the direct control of the vaccination program and probably more related to exchange rate and price, the total number of sheep does effect the project viability. From a purely economic point of view (not including management issues or economies of scale for vaccine production) the number exported could be reduced to approximately 2.4 million head (Table 4) and remain viable, anything less than this would be too high a cost. This is due to the fixed costs; registration and equipment, which needs to be paid irrespective of the number of sheep exported.

It is useful to interpret the impact of the number of sheep vaccinated as potentially representing options where not every animal may be vaccinated (seasonal vaccination for example). The figure of 2.4 million animals represents about 65% of the total of 3.712 million sheep exported from Australia in 2007.

4.2.2.1.7 Vaccine cost

It is expected that the costs of one dose of Salmonella vaccine will cost $0.12/head. If this cost were to rise to $0.143/head or higher, (assuming other inputs remain at expected values) the vaccination program may not be viable (Table 4).

4.2.2.1.8 Vaccine registration cost

This process is assumed to take two years with these costs being incurred in the first year. If the cost of this process was more expensive than expected it could cause a viability issue. If the costs increased to more than $1.025m then it may not be viable on financial grounds. Once again this is a threshold that needs to be considered when negotiating with producers.

4.2.2.1.9 Total vaccination equipment costs at feedlots

Equipment is not purchased until the program is ready to begin. Therefore, these costs are not borne until Year 3. These costs are also estimated at $500,000. If the cost was to blow out to $1.08m then it may threaten the viability of the program. There are no equipment maintenance costs included as a separate item in the cash-flow. These are included in the individual dose rate.

5 Success in achieving objectives

This report meets all of the objectives. It is expected that his report will be circulated amongst industry stakeholders for review and feedback.

6 Impact on meat and livestock industry

The report describes benefits and costs over a 10 year period. It is important to note that the vaccine under consideration in this report is not yet registered in Australia and that there will be requirements for further research in order to finalise development of a vaccine and provide support for an application for registration. As a result there is no immediate impact of the vaccination strategy. The immediate implication of proceeding with the development of a vaccine is associated with costs of research and development, registration and commercial development and marketing of a vaccine.

Within ten years the economic modelling provides estimates of a BCR of 1:1.13.

Intangible benefits are also considered to be important and include additional beneficial impacts on sheep health, welfare and performance and associated improvement in public perceptions concerning the live export trade.

7 Conclusions and recommendations

This report describes current knowledge concerning salmonellosis in live export sheep and about vaccines that are either available or under development as options for reducing the occurrence and severity of salmonellosis.

Salmonellosis and inanition have remained as the most important contributing causes of mortality in live export sheep over the past two decades. Vaccination is one of a number of possible strategies that offers potential to reduce the impact of Salmonella infections as a cause of morbidity and mortality in export sheep. There are a number of challenges associated with developing and implementing a vaccination program. There is no vaccine currently available and registered for this use in Australia. Practices such as short lead-times for sourcing sheep for export shipments, geographic dispersal of source properties with associated difficulties in accessing sheep, large-scale of the export system, potential for heavy exposure to Salmonella in the assembly yards and short assembly feedlot periods, all make it difficult to design and implement an effective vaccination program.

DNA Adenine methylase (DAM) attenuated Salmonella vaccines offer a number of advantages over alternative vaccines for Salmonella that appear to make DAM vaccines the preferred option for further development. While research results to date are based on preliminary data, the results are very promising indeed. DAM vaccines provide rapid onset of effective immunity (within 24 hours of administration), can be delivered orally in drinking water, appear to be safe, are more effective than other vaccines particularly killed bacterins, and offer homologous and heterologous protection. This combination of characteristics is not present within any alternative product and makes the DAM vaccine the only serious candidate vaccine under consideration in this report.

An economic model based on benefit-cost approaches has been developed to allow assessment in monetary terms of the impact of vaccination against Salmonella in sheep entering the live export trade. Economic models are based on assumptions and estimates about losses caused by the disease(s) of interest and the costs and effectiveness of the therapies being considered. The approach allows assessment of the benefits of vaccination expressed as return per dollar spent.

Estimation of costs has involved a detailed understanding of the processes involved in the export industry and valuation of various materials, components, and activities. Costs of vaccination have incorporated estimation of the costs of final research and development and registration of a vaccine as well as estimation of production and marketing costs of a vaccine product. All of these components have been incorporated into one overall estimate and have not been itemised separately. Given the relatively small market size for application of this vaccine (export sheep industry and possibly additional sales into a small sheep feedlot industry), the production option that is considered most likely to be successful is through custom production of small quantities of vaccine under contract by an organisation such as the Victorian DPI. This approach is suited to smaller-scale production and allows avoidance of over-production. Custom production of an oral vaccine that can be made in bulk is also considered to avoid costs associated with production of vaccine in single or multi-dose vials and avoids administration costs associated with equipment (needles, vaccinators) and handling of individual animals.

Benefit-cost analyses generally incorporate discounting of future benefits and costs to express summary information in terms of net present value (NPV) to allow effective comparisons of options that may incur costs or benefits at differing proportions in different years.

All economic models depend heavily on validity of input assumptions and parameters. In this case the development of the model has been aided considerably by detailed research into morbidity and mortality in the live export trade conducted by the authors (LIVE.123) and by others as reported in the literature review component of this report. Consultation with industry stakeholders has also been used to discuss assumptions and parameter values.

The findings of the economic modelling indicate that vaccination is economically viable, dependent on the input assumptions used in the model. This conclusion is based on the finding of a positive NPV meaning that the present value of returns over 10 years is greater than the present value of costs over the same time frame. The same output can be expressed as a benefit/cost ratio (BCR) which is simply the ratio of the present value of benefits to the present value of costs. The estimated BCR for Salmonella vaccination is 1.13 to 1 meaning that for every $1 invested in the program the expected return would be $1.13.

A variety of sensitivity analyses were then performed involving varying influential input parameters by 25% of their expected values in both directions (negative or worst case, and positive or best case). Sensitivity analyses allowed identification of the five most influential input parameters as sale price, exchange rate, Salmonella mortalities on boats, reduction in Salmonella mortalities due to vaccination, and the cost of the vaccine (unit cost). All five influential parameters have the potential to drive the model into a negative 10 year NPV (financially non-viable) when input values were changed in a negative direction.

While industry level estimation of economic outputs such as NPV, BCR and IRR are positive when input parameters are set at expected values, the gross margin per sheep is very low and is estimated to be $0.06/head (gross margin of $16.79/head for unvaccinated animals and $16.85/head for vaccinated animals). Given the size of the export industry in terms of numbers of animals moved per annum even a small per unit benefit has the potential to translate into meaningful values over the course of one or more years. At an industry level the investment in developing and implementing vaccination is considered to be a low risk strategy with a moderate to high likelihood of returning a positive economic benefit.

The economic modelling described in the current report has not incorporated costs and benefits associated with sale of vaccine into components of the sheep industry separate to the live export trade, particularly intensive sheep enterprises and sheep feedlots where product is destined for markets other than export. The decision not to incorporate these segments of the industry into the current modelling was based on a need to restrict the assessment to the live export industry since this was identified as the principal market and the primary decision making process was felt to be constrained to the live export industry. This also meant that a decision on whether to progress with vaccination or not could be made by the export industry based on export-associated benefits and costs. If a Salmonella vaccine were registered and available it is considered likely that sales would be made into segments of the sheep industry unrelated to the live export trade. The impact is therefore assessed as positive but very difficult to quantify.

The economic modelling described in this report has only considered benefits and costs that could be quantified in dollar values. There are other benefits and costs that may be difficult to quantify. These are termed **intangibles**. There is a considerable body of literature that relates to assessment of intangibles and assignment of some form of utility or subjective monetary value to intangibles to allow them to be directly incorporated in economic modelling techniques such as BCA. In this case the major intangibles were identified as those relating to public perceptions about the live export trade.

The live export trade has received adverse publicity over concerns related to animal welfare and including factors influencing morbidity and mortality in export sheep. A variety of strategies have already been implemented in the export industry to ensure that standards of care and welfare are maintained. Any reduction in morbidity and mortality that can be achieved through implementation of an intervention such as Salmonella vaccination is likely to have a strong beneficial impact on perceptions of the trade because of reduced risk (both likelihood and consequence or severity) of salmonellosis. Vaccination against Salmonella is considered highly likely to reduce the incidence of mortality due to Salmonella and also reduce the occurrence of large-scale spikes of outbreaks of disease and mortality due to Salmonella infection.

Ongoing mortality due to Salmonella and particularly outbreaks or spikes of elevated mortality rate in particular shipments, have the potential to result in strident criticism of the industry and renewed calls for live export to be stopped or modified in some way. The impact of such adverse events are far greater than can be summarised through economic impacts of losses associated with morbidity and mortality. An unlikely but potentially possible impact of such events is complete closure of the trade. The value of implementing measures to minimise the risk of such adverse events as closure of the entire trade is self-evident but difficult to quantify.

There may be additional animal welfare benefits other than reduced mortality associated with salmonellosis. These relate to reduced subclinical and clinical morbidity either due to salmonellosis or to other conditions that may occur in sheep affected to some extent by salmonellosis. These effects are likely to have benefits on animal welfare and performance but have not been explicitly incorporated into the modelling because of difficulties in developing assumptions and estimating parameter values.

A more complete economic analysis incorporating monetary values for intangibles may be appropriate when considering decisions to invest in Salmonella vaccination versus investment in some other aspects of animal health associated with live export in order to allow comparisons based on both tangible or estimable and intangible impacts. Such methods are dependent on development of valid and reliable methods of assigning economic values to intangibles. A range of other research referred to in the literature review component of this report has identified salmonellosis and inanition as the major animal health problems associated with live sheep export. These findings provide support for a decision to progress development and implementation of Salmonella vaccination.

The current report indicates a small positive gross margin per sheep and a relatively small positive NPV over 10 years as a result of vaccination against Salmonella. The fact that the current report indicates a positive economic effect of vaccination over a 10 year period is particularly favourable when intangibles are considered. Even in the case where economic modelling returns borderline or low-value negative NPVs, there is believed to be a strong case for arguing that the beneficial impact of intangibles might influence a decision to proceed with vaccination strategies because of the overall impact on the trade. If vaccination has sufficient impact on mortality risk to minimise the risk of severe outbreaks of salmonellosis associated with large-scale mortality, then vaccination may avoid the risk of closure of the entire export trade as a result of adverse public and political pressure. While this may be considered to be an extreme outcome with low likelihood of occurring, the scale of the consequence is such that considerable effort may be justified to avoid that outcome. It is the opinion of the authors that the very small positive economic outputs described in the economic model – when considered in isolation - do not provide sufficient evidence of positive economic return to warrant investment in further development of a vaccine. However, when the value of intangible impacts is considered in conjunction with the small positive economic impact, the combined effect is sufficient to warrant further research and development with a view to registration of an orally active Salmonella vaccination for use in export sheep.

It is acknowledged that there is a requirement for further research and development of a vaccine based on the DAM attenuated vaccine discussed in this report. Such developments are necessary even before a final decision can be made on whether a registered product is a feasible and practical possibility and should provide invaluable information on efficacy and safety for an oral-administered vaccine under Australian conditions.

One of the authors (J House) is actively involved in ongoing research involving development of a S. Typhimurium challenge model in sheep and in further development and efficacy testing of orally administered DAM vaccines to sheep. Preliminary results are promising and should further inform progression of the recommendations of this report.

The report supports a recommendation to proceed with research and development of a vaccine against Salmonella that can be delivered orally through drinking water.

8 Bibliography

1. Associates H. The Live Export Industry: Value, Outlook and Contribution to the Economy, LIVE.314 Meat and Livestock Australia. 2006.
2. Norris R, Norman G. National Livestock Export Industry Shipboard Performance Report 2007, Meat & Livestock Australia. *Live246* 2008.
3. Makin K, House JK, Perkins N, Walker K, Curran G. Investigating Mortality in Sheep and Lambs Exported Through Adelaide and Portland. *Live123, Meat and Livestock Australia* 2008.
4. Higgs A, Norris R. Season, age and adiposity influence death rates in sheep exported by sea. *Australian Journal of Agricultural Research* 1991;42:205-214.
5. Norris R, Norman G. Mortality and morbidity risk factors for livestock during sea transport from Australia. Live.216. Sydney, Meat & Livestock Australia and LiveCorp. 2003.
6. Norris RT, Richards RB, Dunlop RH. An epidemiological study of sheep deaths before and during export by sea from Western Australia. *Aust Vet J* 1989;66:276-279.
7. Richards RB, Norris RT, Dunlop RH, McQuade NC. Causes of death in sheep exported live by sea. *Aust Vet J* 1989;66:33-38.
8. Kelly AP. Mortalities in sheep transported by sea. Faculty of Veterinary Science. Melbourne, University of Melbourne. PhD Thesis Dissertation. 1996.
9. Blood DC, Studdert VP. Bailliere's Comprehensive Veterinary Dictionary, Bailliere Tindall London. 1999.
10. Norris RT, Richards RB. Deaths in sheep exported by sea from Western Australia--analysis of ship Master's reports. *Aust Vet J* 1989;66:97-102.
11. Richards RB, Hyder MW. Seasonal and metabolic factors may be responsible for deaths in sheep exported by sea. *Australian Journal of Agricultural Research* 1991;42:215-226.
12. Higgs A, Norris R. Mortality of sheep exported by sea: evidence of similarity by farm group and of regional differences. *Aust Vet J* 1999;77:729-733.
13. Kelly. Epidemiologic Studies into Salmonellosis in the live sheep trade. *PhD Thesis*:146 - 201.
14. Higgs AR, Norris RT, Richards RB. Epidemiology of salmonellosis in the live sheep export industry. *Aust Vet J* 1993;70:330-335.
15. More S. Salmonellosis control and best practice in live export feedlots. *Live 112* 2002.
16. Wathes CM, Zaidan WA, Pearson GR, Hinton M, Todd N. Aerosol infection of calves and mice with Salmonella typhimurium. *Vet Rec* 1988;123:590-594.
17. Nazer AH, Osborne AD. Experimental Salmonella dublin infection in calves. *Br Vet J* 1977;133:388-398.
18. Hardman PM, Wathes CM, Wray C. Transmission of salmonellae among calves penned individually. *Vet Rec* 1991;129:327-329.
19. Li H, McFarlane RG, Wagner J. Vaccination of pregnant ewes against infection with *Salmonella Brandenburg*. *N Z Vet J* 2005;53:416-422.
20. Robinson RA. Salmonella excretion by sheep following yarding. *N Z Vet J* 1967;15:24-25.
21. Begg AP, Walker KH, Love DN, Mukkur TK. Evaluation of protection against experimental salmonellosis in sheep immunised with 1 or 2 doses of live aromatic-dependent Salmonella typhimurium. *Aust Vet J* 1990;67:294-298.
22. Mukkur TK, Walker KH, McDowell GH. Passive immunisation of neonatal lambs via colostrum and milk of ewes previously immunised with live attenuated Salmonella typhimurium protects neonatal lambs from experimental salmonellosis. *Comp Immunol Microbiol Infect Dis* 1998;21:327-336.

23. Mukkur TK, Walker KH, Stocker BA. Generation of aromatic-dependent Salmonella havana and evaluation of its immunogenic potential in mice and sheep. *Vet Microbiol* 1991;29:181-194.
24. Grau FH, Brownlie LE, Smith MG. Effects of food intake on numbers of salmonellae and *Escherichia coli* in rumen and faeces of sheep. *J Appl Bacteriol* 1969;32:112-117.
25. Brownlie LE, Grau FH. Effect of food intake on growth and survival of salmonellas and Escherichia coli in the bovine rumen. *J Gen Microbiol* 1967;46:125-134.
26. Chambers PG, Lysons RJ. The inhibitory effect of bovine rumen fluid on *Salmonella typhimurium*. *Research Veterinary Science* 1979;26:273-276.
27. Mattila T, Frost AJ, D OB. The growth of Salmonella in rumen fluid from cattle at slaughter. *Epidemiol Infect* 1988;101:337-345.
28. Warnecke T, Gill RT. Organic acid toxicity, tolerance, and production in Escherichia coli biorefining applications. *Microbial Cell Factories* 2005;4.
29. Lawhon SD, Maurer R, Suyemoto M, Altier C. Intestinal short-chain fatty acids alter Salmonella typhimurium invasion gene expression and virulence through BarA/SirA. *Mol Microbiol* 2002;46:1451-1464.
30. Durant JA, Corrier DE, Ricke SC. Short-chain volatile fatty acids modulate the expression of the hilA and invF genes of Salmonella Typhimurium. *J Food Prot* 2000;63:573-578.
31. Fernandez-Rubio C, Ordonez C, Abad-Gonzalez J, et al. Butyric acid-based feed additives help protect broiler chickens from Salmonella Enteritidis infection. *Poult Sci* 2009;88:943-948.
32. Papezova K, Gregorova D, Jonuschies J, Rychlik I. Ordered expression of virulence genes in Salmonella enterica serovar Typhimurium. *Folia Microbiol (Praha)* 2007;52:107-114.
33. Frost AJ, D OB, Samuel JL. The isolation of Salmonella spp from feed lot cattle managed under different conditions before slaughter. *Aust Vet J* 1988;65:224-225.
34. Grau FH, Brownlie LE, Roberts EA. Effect of some preslaughter treatments on the *Salmonella* population in the bovine rumen and faeces. *J Appl Bacteriol* 1968;31:157-163.
35. De Jong H, Ekdahl MO. Salmonellosis in calves - The effect of dose rate and other factors on transmission. *New Zealand Vet J* 1965;13:59-67.
36. Brennan FR, Oliver JJ, Baird GD. Differences in the immune responses of mice and sheep to an aromatic-dependent mutant of Salmonella typhimurium. *J Med Microbiol* 1994;41:20-28.
37. Kerslake JI, Perkins NR. Salmonella Brandenburg: case-control survey in sheep in New Zealand. *N Z Vet J* 2006;54:125-131.
38. Hunter AG, Peek IS. Vaccination control of an outbreak of *Salmonella typhimurium* infection in suckler cows and calves. *British Vet J* 1977;133:239-244.
39. Lax AJ, Barrow PA, Jones PW, Wallis TS. Current perspectives in salmonellosis. *Br Vet J* 1995;151:351-377.
40. Baljer G, Hoerstke M, Dirksen G, Mayr A. Local immunization processes in the small intestine of calves after experimental infection with salmonellae. *Fortschritte der Veterinarmedizin* 1983:62-69.
41. Baljer G, Hoerstke M, Dirksen G, Sailer J, Mayr A. Efficacy of a local and/or parenteral immunization against salmonellosis in calves with inactivated vaccines. *J Vet Med, B Infectious Diseases, Immunology, Food Hygiene, Veterinary Public Health* 1986;33:206-212.
42. Collins FM. Effect of adjuvant on immunogenicity of a heat-killed Salmonella vaccine. *J Infect Dis* 1972;126:69-76.
43. Kenny K, Herzberg M. Early antibody response in mice to either infection or immunization with Salmonella typhimurium. *J Bacteriol* 1967;93:773-778.

44. Gupta JD, Reed CE. Amount, class and specificity of antibody to the lipopolysaccharide of Salmonella enteritidis after immunization by various schedules. *Int Arch Allergy Appl Immunol* 1971;40:256-263.
45. Randow F, Syrbe U, Meisel C, et al. Mechanism of endotoxin desensitization: involvement of interleukin 10 and transforming growth factor beta. *J Exp Med* 1995;181:1887-1892.
46. Fahmi H, Chaby R. Desensitization of macrophages to endotoxin effects is not correlated with a down-regulation of lipopolysaccharide-binding sites. *Cell Immunol* 1993;150:219-229.
47. Matsuura M, Galanos C. Induction of hypersensitivity to endotoxin and tumor necrosis factor by sublethal infection with Salmonella typhimurium. *Infect Immun* 1990;58:935-937.
48. Mastroeni P, Villarreal-Ramos B, Harrison JA, Demarco de Hormaeche R, Hormaeche CE. Toxicity of lipopolysaccharide and of soluble extracts of Salmonella typhimurium in mice immunized with a live attenuated aroA Salmonella vaccine. *Infect Immun* 1994;62:2285-2288.
49. Wilson GS. The hazards of immunization, p265-280 Athlone Press, London. 1967.
50. Robertsson JA, Lindberg AA, Hoiseth S, Stocker B. *Salmonella typhimurium* infection in calves: protection and survival of virulent challenge bacteria after immunization with live or inactivated vaccines. *Infect Immun* 1983;41:742-750.
51. Smith HW. The immunization of mice, calves, and pigs against *Salmonella dublin* and *Salmonella cholerae-suis* infections. *J Hygiene* 1965;63:117-135.
52. Cameron CM, Fuls W. Immunization of mice and calves against *Salmonella dublin* with attenuated live and inactivated vaccines. *Onderstepoort J Vet Res* 1976;43:31-37.
53. Baljer G, Hoerstke M, Dirksen G, et al. Comparative studies of the effectivity of oral immunization with heat-inactivated and live, avirulent (Gal E-) *S. typhimurium* bacteria against salmonellosis in calves. *Zentralbl Veterinarmed [B]* 1981;28:759-766.
54. Smith BP, Reina-Guerra M, Stocker BA, Hoiseth SK, Johnson E. Aromatic-dependent *Salmonella dublin* as a parenteral modified live vaccine for calves. *Am J Vet Res* 1984;45:2231-2235.
55. Meyer H, Koch H, Methner U, Steinbach G. Vaccines in salmonellosis control in animals. *Int J Med Microbiol Virol Parasitol Infect Dis* 1993;278:407-415.
56. Jones PW, Dougan G, Hayward C, et al. Oral vaccination of calves against experimental salmonellosis using a double aro mutant of *Salmonella typhimurium*. *Vaccine* 1991;9:29-34.
57. Sigwart DF, Stocker BA, Clements JD. Effect of a purA mutation on efficacy of Salmonella live-vaccine vectors. *Infect Immun* 1989;57:1858-1861.
58. Stocker BA. Auxotrophic Salmonella typhi as live vaccine. *Vaccine* 1988;6:141-145.
59. Mukkur TKS, McDowell GH, Stocker BAD, Lascelles AK. Protection against experimental salmonellosis in mice and sheep by immunisation with aromatic-dependent *Salmonella typhimurium*. *J Med Microbiol* 1987;24:11-19.
60. Brown RF, Stocker BA. Salmonella typhi 205aTy, a strain with two attenuating auxotrophic characters, for use in laboratory teaching. *Infect Immun* 1987;55:892-898.
61. McFarland WC, Stocker BA. Effect of different purine auxotrophic mutations on mouse-virulence of a Vi-positive strain of Salmonella dublin and of two strains of Salmonella typhimurium. *Microb Pathog* 1987;3:129-141.
62. Mukkur TK, Inman FP. Relative importance of Salmonella-specific antibody isotypes in phagocytosis of Salmonella typhimurium by ovine mammary neutrophils. *Res Vet Sci* 1989;46:153-159.
63. Mukkur TK, Stocker BA, Walker KH. Genetic manipulation of Salmonella serotype Bovismorbificans to aromatic-dependence and evaluation of its vaccine potential in mice. *J Med Microbiol* 1991;34:57-62.

64. Mukkur TK, Walker KH. Development and duration of protection against salmonellosis in mice and sheep immunised with live aromatic-dependent Salmonella typhimurium. *Res Vet Sci* 1992;52:147-153.
65. Mukkur TK, Walker KH, Baker P, Jones D. Systemic and mucosal intestinal antibody response of sheep immunized with aromatic-dependent live or killed Salmonella typhimurium. *Comp Immunol Microbiol Infect Dis* 1995;18:27-39.
66. Mukkur TK, Walker KH, Jones D, Wronski E, Love DN. Immunizing efficacy of aromatic-dependent Salmonella dublin in mice and calves. *Comp Immunol Microbiol Infect Dis* 1991;14:243-256.
67. Heithoff DM, Enioutina EY, Bareyan D, Daynes RA, Mahan MJ. Conditions that diminish myeloid-derived suppressor cell activities stimulate cross-protective immunity. *Infect Immun* 2008;76:5191-5199.
68. Heithoff DM, Enioutina EY, Daynes RA, et al. Salmonella DNA adenine methylase mutants confer cross-protective immunity. *Infect Immun* 2001;69:6725-6730.
69. Dueger EL, House JK, Heithoff DM, Mahan MJ. Salmonella DNA adenine methylase mutants elicit protective immune responses to homologous and heterologous serovars in chickens. *Infect Immun* 2001;69:7950-7954.
70. Dueger EL, House JK, Heithoff DM, Mahan MJ. Salmonella DNA adenine methylase mutants prevent colonization of newly hatched chickens by homologous and heterologous serovars. *Int J Food Microbiol* 2003;80:153-159.
71. Dueger EL, House JK, Heithoff DM, Mahan MJ. Salmonella DNA adenine methylase mutants elicit early and late onset protective immune responses in calves. *Vaccine* 2003;21:3249-3258.
72. Mohler VL, Heithoff DM, Mahan MJ, et al. Cross-protective immunity conferred by a DNA adenine methylase deficient Salmonella enterica serovar Typhimurium vaccine in calves challenged with Salmonella serovar Newport. *Vaccine* 2008;26:1751-1758.
73. Mohler VL, Heithoff DM, Mahan MJ, House JK. Cross-protective immunity in calves conferred by a DNA adenine methylase deficient Salmonella enterica serovar Typhimurium vaccine. . *Vaccine* 2006;24 1339-1345.
74. Heithoff DM, Sinsheimer RL, Low DA, Mahan MJ. An essential role for DNA adenine methylation in bacterial virulence. *Science* 1999;284:967-970.
75. Cooper GL, Venables LM, Woodward MJ, Hormaeche CE. Vaccination of chickens with strain CVL30, a genetically defined Salmonella enteritidis aroA live oral vaccine candidate. *Infect Immun* 1994;62:4747-4754.
76. Foster N, Lovell MA, Marston KL, et al. Rapid Protection of Gnotobiotic Pigs against Experimental Salmonellosis following induction of Polymorphonuclear Leukocytes by Avirulen *Salmonella enterica*. *Infect Immun* 2003;71:2182-2191.
77. Maskell DJ, Hormaeche CE, Harrington KA, Joysey HS, Liew FY. The initial suppression of bacterial growth in a Salmonella infection is mediated by a localized rather than a systemic response. *Microb Pathog* 1987;2:295-305.
78. Nauciel C, Espinasse-Maes F. Role of gamma interferon and tumor necrosis factor alpha in resistance to Salmonella typhimurium infection. *Infect Immun* 1992;60:450-454.
79. Schafer R, Eisenstein TK. Natural killer cells mediate protection induced by a Salmonella aroA mutant. *Infect Immun* 1992;60:791-797.
80. Dueger EL, House JK, Heithoff DM, Mahan MJ. Salmonella DNA adenine methylase mutants elicit protective immune responses to homologous and heterologous serovars in chickens. *Infect Immun* 2001;69:7950-7954.

81. Heithoff DM, Sinsheimer RL, Low DA, Mahan MJ. An essential role for DNA adenine methylation in bacterial virulence. *Science* 1999;284:967-970.
82. Mastroeni P, Chabalgoity JA, Dunstan SJ, Maskell DJ, Dougan G. Salmonella: Immune responses and vaccines. *Vet J* 2001;161:132-164.
83. Bouzoubaa K, Nagaraja KV, Newman JA, Pomeroy BS. Use of membrane proteins from Salmonella gallinarum for prevention of fowl typhoid infection in chickens. *Avian Dis* 1987;31:699-704.
84. Charles SD, Nagaraja KV, Sivanandan V. A lipid-conjugated immunostimulating complex subunit vaccine against Salmonella infection in turkeys. *Avian Dis* 1993;37:477-484.
85. House JK. Detection and prevention of Salmonella infections in dairy cattle. *Thesis Dissertation, University of Califronia, Davis* 1997.
86. Clarke M. Live Export, business Plan. Registration of the aromatic attenuated Salmonella vaccine in the live sheep trade, Project Code: LIVE.007B, Meat and Livestock Australia, August 2004. 2004.