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Escherichia coli O157 vaccination scoping report

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Executive summary

Several human outbreaks of *Escherichia coli* 0157:H7 associated with consumption of hamburger meat have occurred in the USA over the past few years. Recently two vaccines (Econiche™ *Escherichia coli* bacterial extract vaccine – Bioniche Life Sciences; E. coli Bacterial Extract Vaccine – Epitopix) have been approved for use in cattle in the USA to reduce the shedding of *E. coli* 0157:H7. There is concern in the Australian industry that use of a vaccine to reduce shedding of *E. coli* 0157:H7 will become a mandatory requirement for manufacturing beef to enter the USA.

The aim of this scoping study was to review the available scientific information on the two *E. coli* 0157:H7 vaccines approved for use in cattle in the USA and determine the likely effectiveness of these in Australian cattle, registration requirements and the likelihood of vaccine uptake in Australia. The roles Industry could take associated with the availability of these vaccines within Australia were also examined.
The project was a desktop review of the scientific literature using CAB, Medline and Pubmed. Internet searches were conducted to source information on the two North American vaccine manufacturers. Searches were also made of the APVMA website to determine likely registration requirements for the two vaccines within Australia.

The Econiche™ vaccine has been trialled in five studies in feedlot cattle in the USA using one-, two- or three-dose vaccination protocols and been found to reduce colonization of cattle with *E. coli* O157:H7 and faecal shedding of the bacteria, with best results achieved using the three-dose vaccination protocol. Vaccination of all animals within a pen reduced hide contamination with *E. coli* O157:H7 compared to unvaccinated cattle housed in separate pens, whereas comingling vaccinated and unvaccinated cattle in the same pen did not reduce hide contamination.

The E. coli Bacterial Extract Vaccine has been trialled in two commercial feedlot studies in the USA. Vaccinated cattle were less likely to shed *E. coli* O157:H7 in faeces or have E*. coli* O157:H7 isolated from recto-anal or hide swabs than placebo-vaccinated calves. The faecal concentration of *E. coli* O157:H7 was also reduced in vaccinated cattle. Administration of the vaccine to heifers known to be shedding *E. coli* O157:H7 reduced the prevalence and duration shedding of *E. coli* O157:H7 compared to placebo-vaccinated calves, with fewer vaccinated animals classified as high shedders.

Neither vaccine had an effect on productivity measures.
It is likely that at least one of these vaccines will become available for use in cattle in Australia. A commercial vaccine may also emanate from a University of Queensland research project aimed at developing a mucosal vaccine against enterohaemorrhagic *E. coli* or from one of the other research groups active in this area globally.

The prevalence of *E. coli* O157:H7 in Australian cattle is approximately the same as in cattle in North America. This means that Australia cannot use prevalence of colonized/shedding animals as an argument against vaccinating cattle for *E. coli* O157:H7.

Although *E. coli* O157:H7 is not a major public health concern in Australia, it is in North America, a major market for Australian beef, in particular manufacturing beef sourced largely from cull beef and dairy cows. This means that these two groups of cattle are where the efforts to reduce colonization and shedding of *E. coli* O157:H7 should be focussed.

Surveys of the microbiological quality of Australian cattle and sheep carcases, and beef and sheep meat conducted 1993/4, 1998 and 2004 revealed higher isolation rates of *E. coli* O157:H7 from sheep carcases and bulk-packed frozen sheep meat than from cattle carcases and bulk-packed frozen beef. This may be a cause for concern given the growing importance of sheep meat exports to North America.

Registration of a new veterinary vaccine by the APVMA requires evidence to demonstrate the vaccine is manufactured to a standard comparable with the Australian Code of Good Manufacturing Practice for Veterinary Preparations and data to substantiate the efficacy of the vaccine. Specific laboratory and field studies are required to generate the data required to substantiate the efficacy of vaccines, including the duration of protection and the vaccination schedules. Efficacy data generated in Australia are required for the registration of all vaccines intended for use in food-producing animal species, unless there is strong scientific argument that overseas data are applicable to Australia's climatic conditions, genetic stocks and farm management practices.

It is anticipated that registration of either of the Econiche™ vaccine or the E. coli Bacterial Extract Vaccine for use in Australian feedlot cattle will require one confirmatory field study in Australian feedlot cattle. Field studies conducted in Australia will also be required to register either of the vaccines for use in grass-fed beef or dairy cattle in Australia. These studies may need to be conducted in several locations representing the major cattle producing regions of Australia, but this would need to be confirmed with the APVMA.

Registration of a vaccine for use in sheep will require laboratory/pen trials to first determine dose and vaccination protocols, followed by confirmatory field studies.
It is recommended that Industry consider implementing the following in preparation for the availability of an *E. coli* O157:H7 vaccine for use in cattle in Australia:

i. Commission the development of a model based on quantitative risk assessment, marginal economic analysis and available information on the Econiche™ vaccine, the E. coli Bacterial Extract Vaccine and/or hypothetical vaccines to allow break-even points for using *E. coli* O157:H7 vaccines in various sectors of the Australian beef industry (cull dairy cattle, feedlot, grass-fed beef) to reduce the risk of contamination of lots of manufacturing beef destined for the USA.
ii. Commission a modelling study based on available information on the Econiche™ vaccine, the Epitopix E. coli Bacterial Extract Vaccine and/or hypothetical vaccines and prevalence of *E. coli* O157:H7 in Australian dairy cattle to investigate if vaccination strategies focussed on young stock on dairy farms could, by reducing environmental contamination of the farm, reduce shedding by cull cows sent for slaughter. The model should be developed in such a way that it could, when information becomes available, also be used to model the effectiveness of the vaccines on more intensive southern beef properties.
iii. Initiate discussions with Bioniche Life Sciences and Epitopix with the aim of developing a partnership with one of them to ensure that one of their vaccines becomes available for use in Australian cattle and that the vaccine is used in way that will reduce the risk of detection of *E. coli* O157:H7 in manufacturing beef destined for the USA.

It is suggested that the modelling studies are commissioned in the first instance, with the results from these helping to inform the direction of the research program undertaken in partnership with one of the companies.
The potential for registration of either of the vaccines for use in sheep should also be raised with the two companies, including the possibility of a collaborative research program to achieve this.
It is recommended that Industry also consider updating the literature review of neonatal calf scours completed in 2005 to determine if new evidence has emerged on the role of attaching and effacing *E. coli* in the neonatal calf scours syndrome.

Contents

1 Background

Several human outbreaks of *Escherichia coli* 0157:H7 associated with consumption of hamburger meat have occurred in the USA over the past few years. Recently two vaccines have been approved for use in cattle in the USA to reduce the shedding of *E. coli* 0157:H7. There is concern in the Australian industry that use of a vaccine to reduce shedding of *E. coli* 0157:H7 will become a mandatory requirement for manufacturing beef to enter the USA.

The aim of this scoping study was to review the available scientific information on the two *E. coli* 0157:H7 vaccines registered in the USA and determine the likely effectiveness of these in Australian cattle, registration requirements and the likelihood of vaccine uptake in Australia. The roles Industry could take associated with the availability of these vaccines within Australia were also examined.

2 Project Objectives

The objectives of the project were to:

I. review the available scientific information on the two *E. coli* 0157:H7 vaccines registered in the USA
II. estimate the likely uptake of the vaccines by Australian cattle producers
III. determine the likely registration requirements for the vaccines in Australia. This was restricted to review of registration guidelines on the Australian Pesticides and Veterinary Medicines Authority (APVMA) website and did not include direct liaison/communication with the APVMA
IV. examine the various roles Industry could take associated with the availability of these vaccines within Australia.

3 Methodology

The project was a desktop review of the scientific literature using CAB, Medline and Pubmed. Internet searches were conducted to source information on the two North American vaccine manufacturers. Searches were also made of the APVMA website to determine likely registration requirements for the two vaccines within Australia.

4 Results and Discussion

4.1 Available scientific information on *E. coli* O157:H7 vaccines

4.1.1 Rationale for vaccinating cattle

Ruminants are an important reservoir of *E. coli* O157:H7 and numerous outbreaks of disease in humans in North America have been linked to eating foods of bovine origin or contaminated with bovine manure or farm run-off. During the 1990s several studies were carried out in the USA to estimate the prevalence of *E. coli* O157:H7 in beef and dairy cattle (Hancock *et al.* 1994; Faith *et al.* 1996; Hancock *et al.* 1997; Hancock *et al.* 1998; Laegreid *et al.* 1999). These studies suggested a within herd prevalence of cattle shedding *E. coli* O157:H7 of less than 10%. (Refer to Appendix 1 for summary of the findings from these studies.)
However, Elder *et al.(* 2000) reported that the prevalence of *E. coli* O157:H7 in the faeces and on the hides of cattle presented for slaughter in the USA was higher than previously thought, with 28% of animals shedding the bacteria in their faeces and 11% found to have the bacteria on their

hides. Seventy two percent of lots (single sources) of cattle had at least one animal shedding *E. coli* O157:H7 in faeces. This study had used immunomagnetic capture/separation to detect the bacterium, whereas previous prevalence estimates had relied on direct culture. Immunomagnetic capture/separation is more sensitive for detection of *E. coli* O157:H7 (Moxley 2003).

In this pivotal study, Elder *et al.(* 2000) also demonstrated a reduction in carcase contamination with *E. coli* O157:H7 from pre-evisceration (43%) to post-evisceration (18%) to post-processing (2%) and that faecal and hide prevalence on *E. coli* O157:H7 were significantly correlated with carcase contamination. This led the researchers to conclude that in-plant sanitary practices were effective and that there was a role for on-farm control of *E. coli* O157:H7 in live cattle. On-farm control has the added benefit of reducing environmental contamination (Callaway *et al.* 2004) and is consistent with a philosophy of applying controls at all possible points in the food chain (Jordan *et al.* 1999a). Failure of post-slaughter controls to prevent all cases of human food-borne disease is also cited as another reason for implementing pre-slaughter controls for a human food-borne pathogen such as *E. coli* O157:H7 (Jordan *et al.* 1999b; Callaway *et al.* 2004).

Numerous strategies to reduce colonization of cattle with *E. coli* O157:H7 have been tried, including animal management strategies (hygiene, fasting, washing), dietary manipulation, probiotics, antibiotics, bacteriocins/colicins, addition of chlorate to rations, bacteriophages and vaccination (Bach *et al.* 2002; Stevens *et al.* 2002; Callaway *et al.* 2004; Vanselow *et al.* 2005). Two simulation models have been developed to compare the potential effectiveness of these control strategies, one for feedlot cattle (Jordan *et al.* 1999a) and one for dairy cattle (Vosough Ahmadi *et al.* 2007).

Control strategies compared in the feedlot cattle model included vaccination[1], test and sort (with infected cattle slaughtered at the end of the day), duration of fasting (11-20 hours) and washing to remove dags (Jordan *et al.* 1999b). Of these, vaccination (or use of another agent that reduces shedding of *E. coli* O157:H7 in faeces) was found to be the most effective at reducing carcase contamination.

Control strategies compared in the dairy model included vaccination[2], dietary modification, probiotics (colicin) and improved hygiene, singly or in combination (Vosough Ahmadi *et al.* 2007). Baseline prevalence was assumed to be 5.02% for lactating cows and within-herd prevalence 13.96%. Improved hygiene in all groups plus one other control option was found to be most effective at reducing prevalence in lactating cows. Implementing a single intervention in the whole herd or just in young stock was second most effective. Singly, vaccination, dietary modification or probiotics (colicin) were more effective than improved hygiene.

In 2009, researchers at the United States Department of Agriculture published a risk assessment tool for evaluating the use of a hypothetical *E. coli* O157:H7 vaccine used in cattle to prevent illness in humans (Withee *et al.* 2009). A combination of quantitative risk assessment, marginal economic analysis and estimated parameters for a hypothetical vaccine based on available literature were used to estimate break-even points for vaccinating cattle, including the minimum effectiveness of a vaccine that still allows it to be cost-effective at a fixed price, the maximum price allowable at a fixed effectiveness and optimal levels of vaccine use at a fixed price and effectiveness.

This model is not directly applicable to Australia, where the aim of vaccination would be to prevent contaminated lots of manufacturing beef being exported to the USA, rather than cases of

[1] Theoretical vaccine assumed to reduce within herd prevalence to 20% of baseline level and concentration of *E. coli* O157:H7 in faeces by 50%, and to be used regardless of herd status.
[2] Based on results published by Potter *et al.(* 2004)

human illness per se. For example, the model assumed mainly young stock are slaughtered whereas Australian cattle destined to become manufacturing beef for export to North America are primarily cull beef and dairy cows. However, a risk assessment tool based on a similar approach may be useful in deciding how best to use an *E. coli* O157:H7 vaccine in cattle in Australia, for example, the cost effectiveness of vaccinating different classes/numbers of stock to prevent contaminated lots of manufacturing beef being exported to the USA.

4.1.2 Econiche™ *Escherichia coli* Bacterial Extract Vaccine (Bioniche Life Sciences Inc.)

The Econiche™ *Escherichia coli* bacterial extract vaccine was developed in Canada by researchers at the Veterinary and Infectious Disease Organization at the University of Saskatchewan, the Biotechnology Laboratory at the University of British Columbia and Bioniche Life Sciences Inc., a Canadian biopharmaceutical company based in Belleville Ontario[3]. The animal health division of Bioniche has an office in Australia and currently the company markets a limited range of mainly vet-only prescription products within Australia[4]. A representative of the company was recently in Australia and indicated that the company is currently seeking an Australian Quarantine and Inspective Service permit to enable the Econiche™ vaccine to be imported into Australia.

The Econiche™ vaccine contains secreted proteins (Esps and Tir) from broth supernatant of *E. coli* O157:H7 formulated with the adjuvant Emulsigen®-D[5]. Econiche™ is approved for use in cattle in Canada and the USA (conditional USDA licence) and is administered by subcutaneous injection in the neck using a vaccination protocol of three 2 mL doses given at 3 week intervals[6]. The scientific rationale for developing a vaccine containing secreted proteins from *E. coli* O157:H7 was based on the research findings of various groups during the late 1990s and early 2000s, including that:

i. *E. coli* O157:H7 produces a secreted virulence factor Tir that is inserted into the host cell membrane and plays a role in bacterial adherence (DeVinney *et al.* 1999)
ii. humans with disease caused by *E. coli* O157:H7 mount an immune response to virulence factors secreted by *E. coli* O157:H7, with the strongest response against Tir (Li *et al.* 2000). Weaker immune responses are also observed against intimin, a protein on the outer wall of the bacteria that binds to Tir; EspA, a protein that forms filamentous structures on the surface of the bacteria; and EspB, a protein that is inserted into the host cell membrane and cytoplasm
iii. intimin is required for *E. coli* O157:H7 to colonize the gastrointestinal tract of ruminants (Cornick *et al.* 2002; Dean-Nystrom *et al.* 1998)
iv. intimin is needed for *E. coli* O157:H7 to cause attaching and effacing lesions in calves (Dean-Nystrom *et al.* 1998)

[3] www. Bioniche.com
[4] www.apvma.gov.au accessed 12 January 2010.
[5] Emulsigen®-D is an emulsified oil-in-water adjuvant plus immunostimulant (dimethyldioctadecylammonium bromide) that is free of animal origin ingredients (www.mvp-technologies.com). Emulsigen is incorporated in one vaccine registered for use in animals in Australia, Fel-0-VAX-LV-K feline leukemia vaccine for cats (www.apvma.gov.au accessed 12 January 2010).
[6] Econiche™ *Escherichia coli* bacterial extract vaccine technical information brochure
Together, these findings appear to have focussed the North American beef industry and its associated research community on the search for effective vaccines to reduce colonization of cattle with *E. coli* O157:H7

v. epithelial cell explants derived from the colon and rectum of adult cattle developed attaching and effacing lesions following infection with *E. coli* O157:H7, suggesting that infection of the large intestine contributes to development of the carrier state in adult cattle (Baehler and Moxley 2000)
vi. vaccination of sows against intimin protected piglets against intestinal colonization by *E. coli* O157:H7 and prevented the development of attaching and effacing lesions (Dean-Nystrom *et al.* 2002).

Subsequently, a study in a mouse model of *E. coli* O157:H7 colonization demonstrated that subcutaneous or intranasal immunization against Tir and EspA prevented colonization by *E. coli* O157:H7 (Babiuk *et al.* 2008), and a study in adult cattle demonstrated a significant serum antibody response to *E. coli* O157:H7 secreted proteins (Tir, EspA, EspB and intimin) following experimental infection (Bretschneider *et al.* 2007).

Efficacy studies conducted with the Econiche™ vaccine are summarized in Appendix 2.
Using an experimental challenge model in calves, a prototype vaccine induced a serum antibody response against *E. coli* O157:H7, reduced the proportion of calves shedding *E. coli* O157:H7 and the duration and level of shedding (Potter *et al.* 2004). Administration of the prototype vaccine to cattle housed in a research feedlot also reduced the proportion of animals shedding *E. coli* O157:H7 following natural challenge with *E. coli* O157:H7 (Potter *et al.* 2004). However, a prototype vaccine containing formalin inactivated antigens failed to reduce pen prevalence of *E. coli* O157:H7 shedding when trialled in commercial feedlots in western Canada (Van Donkersgoed *et al.* 2005).

The commercial vaccine has been trialled in five studies in feedlot cattle in the USA using one-, two- or three-dose vaccination protocols and been found to reduce faecal shedding of *E. coli* O157:H7 by 33%-73% and the likelihood of colonization of the terminal rectum mucosa by 92-98.3%, with best results achieved using the three-dose protocol (Peterson *et al.* 2007b; Peterson *et al.* 2007a; Smith *et al.* 2008; Smith *et al.* 2009; Moxley *et al.* 2009; Smith *et al.* 2010). Vaccination of all animals within a pen reduced hide contamination with *E. coli* O157:H7 by 55% compared to unvaccinated cattle housed in separate pens, whereas comingling vaccinated and unvaccinated cattle in the same pen did not reduce hide contamination (Smith *et al.* 2010).

No references were found that described the efficacy of the vaccine in grass-fed beef cattle (finishing or cow-calf units) or in dairy cattle. Information on the duration of immunity provided by the vaccine also was not found, but this was not surprising given that all studies were in feedlot cattle about to be slaughtered.

The likelihood of the vaccine to protect cattle against colonization by non-O157 strains (O26:H11, O103:H2, O111:NM) has also been examined, with disappointing results (Asper *et al.* 2007). Cattle vaccinated with secreted proteins from each of the serotypes developed antibodies to secreted proteins of homologous seroptypes, but cross reactivity against Tir and EspA for serotype O157:H7 was not observed. Work on a second generation vaccine that will prevent colonization with non-O157 strains is underway at the University of Saskatchewan[7].

4.1.3 E. coli O157 Bacterial Extract Vaccine (Epitopix LLC.)

The E. coli O157 Bacterial Extract Vaccine was developed in the USA by Epitopix LLC, with researchers from the Kansas State University and West Texas A&M University providing scientific assistance. Epitopix is an American vaccine research and development company based

[7] www.foodprocessing.com.au

in Wilmar Minnesota[8]. In 2000, Epitopix patented a novel technology for manufacturing siderophore and porin receptor (SRP®)-based vaccines[9]. Epitopix does not have an office in Australia and does not market any products in Australia[10], although the company's website indicates that it "values key partnerships to assist in the marketing and distribution of licensed products in the U.S. and world-wide".
The E. coli Bacterial Extract Vaccine contains purified siderophore receptor and porin proteins isolated from a bovine-origin strain of *E. coli* O157:H7 formulated with the Emulsigen® adjuvant (Thornton *et al.* 2009). The vaccine was approved for use in cattle within the USA in 2009 (conditional USDA licence)[11].

In 2004 Epitopix received a conditional USDA license for a Salmonella Newport Bacterial Extract Vaccine, which is also based on their SRP® technology[12].
Siderophores are iron transport proteins found on the outer surface of bacteria. The scientific rationale for developing a vaccine containing siderophore proteins from *E. coli* O157:H7 was based on numerous research findings published on the role of these proteins, including:

i. the role siderophores have in microbes (bacteria and fungi) in scavenging iron, which is usually essential in microbial cells (Neilands 1995)
ii. that siderophores are not found in plants and animals, which have their own pathways for taking up iron (Neilands 1995)
iii. that siderophores have a role in the virulence of several pathogenic bacteria, including *E. coli,* in humans and animals (Neilands 1995)
iv. that in a mouse model of ascending *E. coli* urinary tract infection vaccination against the siderophore receptor protein protected against renal infection (Russo *et al.* 2003).

Efficacy studies conducted with the E. coli Bacterial Extract Vaccine are summarized in Appendix 3.

In an experimental challenge infection model in calves, the vaccine produced an increased serum antibody response to *E.* coli O157:H7 SRP compared to placebo-vaccinated calves and reduced the number of calves shedding *E. coli* O157:H7 following challenge infection, with a trend to reduced faecal concentration of the bacteria (Thornton *et al.* 2009). When administered to heifers known to be shedding *E. coli* O157:H7, the vaccine increased serum antibody response to *E. coli* O157:H7 SRP and reduced the prevalence and duration of heifers shedding *E. coli* O157:H7 compared to placebo-vaccinated calves, with fewer vaccinated animals classified as high shedders (Fox *et al.* 2009).

The vaccine has also been trialled in two commercial feedlot studies in the USA (Thomson *et al.* 2009). Administration of the vaccine had no effect on productivity measures. Vaccinated cattle were less likely to shed *E. coli* O157:H7 in faeces or have *E. coli* O157:H7 isolated from recto-anal or hide swabs than placebo-vaccinated calves. The faecal concentration of *E. coli* O157:H7 was also reduced in vaccinated cattle.

No references were found that described the efficacy of the vaccine in grass-fed beef cattle (finishing or cow-calf units) or in dairy cattle. The duration of immunity produced by the vaccines was not reported in any of the studies, which is not surprising for a vaccine being trialled in feedlot cattle about to be slaughtered.

[8] www.epitopix.com
[9] US patent number 7,371,393
[10] www.apvma.gov.au accessed 13 January 2010
[11] www.agriview.com
[12] www.epitopix.com

No references were found on the likelihood of the vaccine to protect cattle against colonization with other serotypes of *E. coli* O157:H7, although siderophores are relatively conserved within a bacterial species.

4.1.4 Other approaches to vaccinating cattle against *E. coli* O157:H7

Several other approaches to vaccinating cattle to reduce colonization and shedding of *E. coli* O157:H7 are reported in the scientific literature. A brief description of some of these is provided below.

In the late 1990s, the potential of the O157 antigen of *E. coli* O157:H7 was investigated in a mouse model, with mixed results (Conlan *et al.* 1999a; Conlan *et al.* 1999b; Conlan *et al.* 2000). Inoculation of mice with *Salmonella landau*, which naturally expresses the O157 antigen but is not pathogenic for mice, provided partial protection against challenge with *E. coli* O157:H7 (Conlan *et al.* 1999b). Subcutaneous administration of a horse serum albumin - O157 antigen conjugate resulted in variable serum antibody responses and did not protect mice from colonization by *E. coli* O157:H7 (Conlan *et al.* 1999a). Oral administration of the same vaccine mixed with a mucosal adjuvant generated local and systemic immune responses, but also did not protect mice from colonization with *E. coli* O157:H7 (Conlan *et al.* 2000).

Intimin (the protein on the outer wall of the bacteria that binds to Tir inserted into the host cell) has also been investigated as a vaccine antigen ((Judge *et al.* 2004; van Diemen *et al.* 2007; Keller *et al.* 2010). Mice immunized intraperitoneally with purified intimin produced in transgenic tobacco plants and/or fed the transgenic plants developed an intimin-specific serum antibody response and shed *E. coli* O157:H7 for less time following experimental infection that unvaccinated mice (Judge *et al.* 2004). However, calves vaccinated intramuscularly with subunit intimin and/or Efa-1 polypeptide vaccines were not protected from challenge with *E. coli* O157:H7 (van Diemen *et al.* 2007). Recently, a subunit of intimin delivered orally to rabbits using *Vibrio cholera* CVD 103-HgR as a vector was reported to protect rabbits against challenge with *E. coli* O157:H7 (Keller *et al.* 2010).

The utility of purified H7 flagellin as a vaccine antigen has been investigated by researchers in Scotland with promising results obtained following intramuscular injection in calves (McNeilly *et al.* 2008). These researchers, in collaboration with the University of Queensland, are also investigating other bacterial virulence/adhesion factors as potential vaccine antigens (Wells *et al.* 2009). Recently, researchers at the University of Queensland, in collaboration with researchers at the Queensland Department of Employment, Economic Development and Innovation, CSIRO Food Science Australia, Washington State University and the Washington Vaccine Alliance, have been awarded a $A1.94 million Smart Future research grant from the Queensland Government to develop a mucosal vaccine against enterohaemorrhagic *E. coli* in cattle[13].

In December 2009, the USDA announced that two of its researchers had developed candidate vaccines against *E. coli* O157:H7 in cattle and were in the process of seeking patents for these[14].

4.2 Likely uptake of the vaccines by Australian cattle producers

Although *E. coli* O157:H7 has been reported to be pathogenic in neonatal calves (Dean-Nystrom *et al.* 1997), it is largely considered to be part of the normal flora of the gastrointestinal tract of cattle (Bach *et al.* 2002), and vaccination of feedlot cattle to reduce shedding does not result in

[13] http://www.uq.edu.au
[14] www.meatingplace.com

productivity gains (Potter *et al.* 2004; Peterson *et al.* 2007b; Peterson *et al.* 2007a; Fox *et al.* 2009; Thomson *et al.* 2009). This means that improved on-farm productivity is unlikely to be a driving force for adoption of an *E. coli* O157:H7 vaccine on beef cattle properties.

Over the past 10-15 years several studies have reported the prevalence of either Shiga toxin-producing *E. coli* or *E. coli* O157:H7 in Australian beef and dairy cattle (Desmarchelier 1997; Sidjabat-Tambunan and Bensink 1997; Cobbold and Desmarchelier 2000; Hallaran and Sumner 2001; Hornitzky *et al.* 2002; Fegan *et al.* 2004; Fegan *et al.* 2005). The results from these studies are summarized in Appendix 4. Enrichment and immunomagnetic separation were utilized for screening the prevalence of *E. coli* O157:H7 in only two of these studies, both of which were in beef cattle (Fegan *et al.* 2004; Fegan *et al.* 2005). Prevalence of faecal shedding was reported to be 13% in the first study and 10% in the second, similar to reported prevalence of *E. coli* O157:H7 in cattle presented for slaughter in Canada (Van Donkersgoed *et al.* 1999) and the United Kingdom (Chapman *et al.* 1997), but less than that of feedlot cattle reported for slaughter in the USA (Elder *et al.* 2000). (Refer to Appendix 1 for a summary of international prevalence studies.)

Surveys of the microbiological quality of Australian beef have detected *E. coli* O157:H7 on Australian beef carcases but not in frozen bulk-packed meat, with a reduction in detection rates on carcases between 1993/4 and 1998 and similar detection rates in 1998 and 2004 (Vanderlinde *et al.* 1998; Phillips *et al.* 2001a; Phillips *et al.* 2006a). (Refer to Appendix 5 for a summary of these three studies.) Of note, detection rates on Australian sheep carcases and in frozen-bulk packed sheep meat were higher than on beef carcases and in frozen-bulk-packed beef (Vanderlinde *et al.* 1999; Phillips *et al.* 2001b; Phillips *et al.* 2006b), which may be a cause for concern giving increasing sheep meat exports to North America.

Despite the similar prevalence of *E. coli* O157:H7 in Australian and overseas cattle, Australia has experienced only limited human disease associated with *E. coli* O157:H7 and the bacterium is not considered a major food safety risk in Australia (Vanselow *et al.* 2005). Reasons suggested for this are a lack of important virulence factors in Australian *E. coli* O157:H7 strains, environmental factors such as sunlight limiting environmental contamination, human drinking water source and hygiene, abattoir hygiene and a preference for well-cooked ground beef (Vanselow *et al.* 2005). Regardless of the reason, the lack of human cases within Australia means that domestic food safety policy is unlikely to be a driving force for adoption of an E*. coli* O157:H7 vaccine, particularly one that is serotype-specific.

This suggests that the market for a serotype-specific *E. coli* O157:H7 vaccine will be cattle slaughtered for overseas markets, in particular as manufacturing beef destined for North America. Cattle that fit this description include largely cast-for-age beef cows and cull dairy cattle. No information is available on the efficacy of either the Econiche™ or Epitopix E. coli Bacterial Extract vaccine to reduce shedding of in these classes of cattle. Unless the vaccines are effective in these classes of cattle it is difficult to envisage applicability to the Australian cattle industry.

Even if the vaccines are found to reduce shedding by cull cows it is unlikely that beef producers will adopt them unless use becomes mandatory or they are penalized for not doing so (i.e. by processors or a trading scheme). Many beef production systems Australia, particularly in northern Australia, are not compatible with three- or even two-dose vaccination protocols. In addition, cull cows are generally low value animals and the vaccines are unlikely to improve their productivity.

The situation may be different for dairy farms and perhaps more intensive beef properties in southern Australia on which management practices may be more conducive to use of the vaccines. The prevalence of *E. coli* O157:H7 has been studied in Australian dairy cattle (Cobbold and Desmarchelier 2000; Hallaran and Sumner 2001). Cobbold and Desmarchelier (2000) reported that the with-in herd prevalence of *E. coli* O157:H7 in Australian dairy cattle was similar to that of dairy cattle in the northern hemisphere and that 1-14 week old calves were the primary source of *E. coli* O157:H7 on dairy farms. Studies conducted in the northern hemisphere have also identified young dairy cattle (weaned calves and heifers) as shedding high levels of *E. coli* O157:H7 (Hancock *et al.* 1994; Mechie *et al.* 1997; Heuvelink *et al.* 1998). Hallaran and Sumner (2001) reported a lower prevalence of *E. coli* O157:H7 in cull dairy cows than reported overseas, but their survey was conducted during winter months and relied on sampling two cull cows from each of 200 dairy farms, a sampling method that is not well suited to a condition with low with-in herd prevalence such as *E. coli* O157:H7 shedding by adult dairy cows. Both studies relied on PCR rather than immunomagnetic capture to screen samples collected during the surveys for *E. coli* O157:H7.

On dairy farms, a program aimed at encouraging vaccination of young stock may be more successful than a program aimed at vaccinating lactating cows soon to be culled. In the dairy cattle model discussed previously, vaccination of young stock alone was predicted to be as effective at reducing shedding of *E. coli* O157:H7 in the lactating herd as vaccinating the whole herd and more effective than vaccinating lactating cows (Vosough Ahmadi *et al.* 2007). This finding is a reflection of the high shedding rate of *E. coli* O157:H7 in young stock (Hancock *et al.* 1994; Mechie *et al.* 1997; Heuvelink *et al.* 1998; Cobbold and Desmarchelier 2000) and that interventions aimed at reducing this should decrease the overall contamination level on the farm. Attaching and effacing *E. coli*, including *E. coli* O157:H7, are pathogenic in neonatal calves, particularly those less than 3 weeks of age (Dean-Nystrom *et al.* 1997). If either of the vaccines reduced contamination of dairy farms with attaching and effacing *E. coli* and, as a result, helped reduce the incidence neonatal calf diarrhoea (a syndrome caused by viruses, bacteria including *E. coli*, protozoa and environmental

factors), this may help drive adoption of the vaccine. The same may be true for more intensive beef properties in southern Australia, on which neonatal calf diarrhoea is often a substantial problem.

4.3 Likely registration requirements for the vaccines in Australia

New vaccines for use in animals, with the exception of autogenous vaccines, must be registered by the Australian Pesticides and Veterinary Medicines Authority (APVMA) prior to sale in Australia. Imported vaccines must also obtain an import permit from the Australian Quarantine and Inspection Service before the APVMA will register the vaccine.

Registration of a new veterinary vaccine by the APVMA requires evidence to demonstrate the vaccine is manufactured to a standard comparable with the Australian Code of Good Manufacturing Practice for Veterinary Preparations and data to substantiate the efficacy of the vaccine. Specific laboratory and field studies are required to generate the data required to substantiate the efficacy of vaccines, including the duration of protection and the vaccination schedules. Efficacy data generated in Australia are required for the registration of all vaccines intended for use in food-producing animal species, unless there is strong scientific argument that overseas data are applicable to Australia's climatic conditions, genetic stocks and farm management practices.

Information must also be provided on the potential for the vaccine to contaminate the environment and proposed disposal methods for unused or waste product.

New veterinary vaccines are generally exempt from requirements for toxicological and occupational health and safety assessments unless these contain a new adjuvant or another excipient of OHS concern. The Econiche™ vaccine and the Epitopix E. coli Bacterial Extract Vaccine both contain an adjuvant (Emulsigen®) that is used in at least one other veterinary vaccine sold in Australia.

For a vaccine to be used in a food producing species, residues data are usually not required, although consideration of the impact on trade is. The relevant livestock industry is usually given the opportunity to comment on impact on trade.

It may be possible for a company to argue that Australian feedlot cattle are managed much the same as North American feedlot cattle and that, as a result, specific Australian field studies are not required for an *E. coli* O157:H7 vaccine for use in feedlot cattle. However there are differences, particularly in times on feed and the diets fed, and this argument may not be accepted by the APVMA. It will also be easier to market the vaccine if a field study has been conducted in Australia. Therefore, it is anticipated that one confirmatory field study will be required in Australian feedlot cattle, conducted using a similar protocol to the North American studies.

Field studies conducted in Australia will be required to register either of the vaccines for use in grass-fed beef or dairy cattle in Australia. This is because of the lack of published information on use of the vaccines in these classes of stock. Although the vaccine manufacturers may have unpublished information of the use of the vaccines in grass-fed beef or dairy cattle that could be used to support registration, the differences in management practices between Australia and North America mean that Australian studies will be required. For example, the majority of North American dairy cattle are intensively housed, whereas the majority of Australian dairy cattle are grass-fed.

Registration of a vaccine for use in sheep will require laboratory/pen trials to first determine dose and vaccination protocols, followed by confirmatory field studies.

4.4 Roles industry could take associated with the availability of these vaccines within Australia

Roles industry could take associated with the availability of these vaccines include to:

1. do nothing and let market forces determine the availability of either vaccine or a vaccine that may emanate from either the research program at the University of Queensland or another research group. The momentum towards vaccination of North American cattle to reduce shedding of *E. coli* O157:H7 and the research activity in this area globally, including on other serotypes of enterohaemorrhagic *E. coli,* is such that it is considered inevitable that a vaccine against these bacteria will become available for use in Australia
2. commission the development of a model based on a combination of quantitative risk assessment, marginal economic analysis and available information on the Econiche™ vaccine, the E. coli Bacterial Extract Vaccine and/or hypothetical vaccines to investigate the break-even points for *E. coli* O157:H7 vaccines used in cattle (dairy, feedlot and grass-fed beef) to prevent contaminated lots of manufacturing beef being exported to the USA. A similar model has recently been developed by the USDA to investigate the break-even points for a hypothetical *E. coli* O157:H7 vaccine used in cattle to prevent illness in humans (Withee *et al.* 2009)
3. commission a modelling study based on available information on the Econiche™ vaccine, the E. coli Bacterial Extract Vaccine and/or hypothetical vaccines and prevalence of *E. coli* O157:H7 in Australian dairy cattle to investigate if vaccination strategies focussed on

young stock on dairy farms could, by reducing environmental contamination of the farm, reduce shedding by cull cows sent for slaughter and subsequent contamination of lots of manufacturing beef destined for the USA. The model should be developed in such a way that it could, when information becomes available, also be used to model the effectiveness of the vaccines on more intensive southern beef properties

4. initiate discussions with Bioniche Life Sciences and Epitopix with the aim of determining the likelihood and potential timeline for registration of their product in Australia and to provide information on the Australian market
5. initiate discussions with both companies with the aim developing a partnership with one of them to ensure availability of one of the vaccines for use in cattle in Australia. The aim of this would be to assist with collection of information required for registration of the product in Australia, say for feedlot cattle (It is considered likely that this is the market either company would target based on their experiences in North America and because it would be the easiest to gain registration for in the short term.) Given that Bioniche is already active in the Australian market and reportedly investigating an import permit for the Econiche™ vaccine, a partnership with this company is most likely
6. initiate discussions with both companies with the aim developing a partnership with one of them to ensure that one of the vaccines becomes available for use in cattle in Australia and that it will be used in way that will reduce the risk of detection of *E. coli* O157:H7 in manufacturing beef destined for the USA. This option will require research beyond that required simply for registration of either vaccine for use in cattle and would require, at a minimum, the modelling studies described in options 2-3 and a longitudinal study in representative southern beef herds. The discussion should also include the potential for registering one of the vaccines for use in sheep. A comprehensive research program will be required to register a vaccine for use in sheep. Again, given the activities of Bioniche is Australia, a partnership with this company is most likely.

On these options Option 2, 3 and 6 are recommended.
Options 1 and 3 may appear attractive in the short-term because the financial investment by industry is less. However, in the longer term these options may prove to be more costly for industry than a combination of Option 2, 3 and 6 because they could result in a vaccine or vaccines becoming available that target an inappropriate segment of the Australian cattle industry (i.e. feedlot cattle only), imposing a cost on industry with little gain in terms of reducing the risk of *E. coli* O157:H7 contaminating export beef destined for the North America. Option 5 is also likely to result in a vaccine targeted at feedlot beef cattle. During the product registration process, the APVMA usually gives the relevant Industry the opportunity to comment on the potential impact of the new product on trade, which may present an opportunity for Industry to comment on the usefulness or otherwise of an *E. coli* O57:H7 vaccine for use only in feedlot cattle.

It is suggested that the recommended Options be undertaken in two stages. First would be commissioning of the two modelling studies. As part of this it is suggested that industry meet with Bioniche and Epitopix to inform them of the studies and that the aim of these is to determine how their vaccines might best be used in Australian cattle. The companies may have information that could inform the modelling studies and might be prepared to provide this following signing of a confidentiality agreement. Option 6 would follow, with the emphasis of the research work guided by the results of the modelling studies.

A precedent for Option 6 is collaborative approach between Pfizer Animal Health, Animal Health Australia and MLA to register Pfizer's bovine Johne's disease vaccine for cattle in Australia. Pfizer would have conducted research only to register the vaccine in Australia (Ross Henderson, pers comm.), whereas the collaborative approach has ensured that information is generated to

enable use of the vaccine in a way that is compatible with Australia's national bovine Johne's disease control program.

5 Conclusions and Recommendations

The North American beef industry and its associated research community have been focussed on developing effective vaccines to reduce colonization of cattle with *E. coli* O157:H7 for at least 10 years. Two vaccines have recently been licensed for use in cattle in North America. The structure of the North America beef industry has meant that, to date, the research efforts to support commercialization of these vaccines has focused largely on feedlot beef cattle. Both vaccines have been demonstrated to reduce the colonization of the gastrointestinal tract of cattle by *E. coli* O157:H7 and to reduce faecal shedding and hide contamination. One of the vaccines has also been demonstrated to reduce the number of 'super-shedders' within a feedlot pen.
It is likely that at least one of these vaccines will become available for use in cattle in Australia. A commercial vaccine may also emanate from the University of Queensland research project aimed at developing a mucosal vaccine against enterohaemorrhagic *E. coli* or from one of the other research groups active in this area globally.

The prevalence of *E. coli* O157:H7 in Australian cattle is approximately the same as in cattle in North America. This means that Australia cannot use prevalence of colonized/shedding animals as an argument against vaccinating cattle for *E. coli* O157:H7.

Surveys of the microbiological quality of Australian cattle and sheep carcases, and beef and sheep meat conducted 1993/4, 1998 and 2004 revealed higher isolation rates of *E. coli* O157:H7 from sheep carcases and bulk-packed frozen sheep meat than from cattle carcases and bulk-packed frozen beef. This may be a cause for concern given the growing importance of sheep meat exports to North America.

Although *E. coli* O157:H7 is not a major public health concern in Australia, it is in North America, a major market for Australian beef, in particular manufacturing beef sourced largely from cull beef and dairy cows. This means that these two groups of cattle are where the efforts to reduce colonization and shedding of *E. coli* O157:H7 should be focussed.
To help ensure this happens, it is recommended that the Industry consider implementing the following:

iv. Commission the development of a model based on quantitative risk assessment, marginal economic analysis and available information on the Econiche™ vaccine, the E. coli Bacterial Extract Vaccine and/or hypothetical vaccines to allow break-even points for using *E. coli* O157:H7 vaccines in various sectors of the Australian beef industry (cull dairy cattle, feedlot, grass-fed beef) to reduce the risk of contamination of lots of manufacturing beef destined for the USA.

v. Commission a modelling study based on available information on the Econiche™ vaccine, the Epitopix E. coli Bacterial Extract Vaccine and/or hypothetical vaccines and prevalence of *E. coli* O157:H7 in Australian dairy cattle to investigate if vaccination strategies focussed on young stock on dairy farms could, by reducing environmental contamination of the farm, reduce shedding by cull cows sent for slaughter. The model should be developed in such a way that it could, when information becomes available, also be used to model the effectiveness of the vaccines on more intensive southern beef properties.

vi. Initiate discussions with Bioniche Life Sciences and Epitopix with the aim developing a partnership with one of them to ensure that one of their vaccines becomes available for use in cattle in Australia and is used in way that will reduce the risk of detection of *E. coli*

O157:H7 in manufacturing beef destined for the USA. Discussion should also be held on the potential for registering one of the vaccines for use in sheep.

It is suggested that the modelling studies are commissioned in the first instance, with the results from these helping to inform the direction of the research program undertaken in partnership with one of the companies.

It is recommended that Industry consider updating the literature review of neonatal calf scours completed in 2005 to determine if new evidence has emerged on the role of attaching and effacing *E. coli* in the neonatal calf scours syndrome.

6 Appendices

Appendix 1 – Summary of studies reporting the prevalence of *E. coli* O157:H7 in the faeces of cattle from countries other than Australia
Appendix 2 – Summary of published efficacy studies with Econiche™ *Escherichia coli* bacterial extract vaccine (Bioniche Life Sciences Inc.)
Appendix 3 – Summary of published efficacy studies with E. coli Bacterial Extract Vaccine (Epitopix LLC.)
Appendix 4 – Summary of reported studies of the prevalence of *E. coli* O157:H7 in Australian cattle
Appendix 5 – Summary of reported studies of the prevalence of *E. coli* O157:H7 on Australian beef carcases and in frozen bulk-packed beef
Appendix 6 – Reference list

6.1 Appendix 1 – Summary of studies reporting the prevalence of *E. coli* O157:H7 in the faeces of cattle from countries other than Australia

Country	Cattle type	Sample			Test			Prevalence			Reference
		Type	Time	Amount	Enrichment	PCR	Immuno-magnetic separation	Farm/ pen/ lot	Within farm/ pen/ lot	Animal	
Canada	Mixed beef and cull dairy cows	Faecal sample	Year round (12 months), at evisceration	10 g	Yes	No	Yes	-	-	7.5% of 1247 faecal samples; prevalence higher in summer (19.7%) and in yearling cattle (4.9%)	(Van Donkersgoed *et al.* 1999)
New Zealand	Dairy, 371 cull cows from 55 farms	Faecal sample from colon	At slaughter, time of year not provided	25 g	Yes	No	Yes	-	-	2%	(Buncic and Avery 1997)
Serbia	Not provided	Faecal sample	At slaughter, time of year not provided	Not provided	Yes	No	Yes	-	-	2.6%	(Nastasijevic *et al.* 2009)

Country	Cattle type	Sample			Test			Prevalence			Reference
		Type	Time	Amount	Enrichment	PCR	Immuno-magnetic separation	Farm/ pen/ lot	Within farm/ pen/ lot	Animal	
The Netherlands	Dairy	Faecal sample	Autumn	20 g	Yes	Yes	Yes	70% (farm)	0.8-22.4% (range)	-	(Heuvelink *et al.* 1998)
UK	Mixed beef (38.4%), cull dairy (34.6%) and other unspecified (27%)	Swab of rectal faeces	Year round (400 cattle per month), immediately after slaughter	NA	Yes	No	Yes	-	-	15.7% (752 of 4800 cattle); prevalence higher in spring and summer[15]	(Chapman *et al.* 1997)
USA	Dairy cattle (3570 mixed age) from 60 farms; 1412 pastured beef cattle from 25 farms	Swabs from rectum or fresh faeces	On farm, time of year not provided	NA	No – beef Yes – dairy	No	No	Beef – 16% Dairy – 8.3%	-	Beef – 0.71% (10 of 1412 cattle) Dairy - 0.28 % (10 of 3570 cattle)	(Hancock *et al.* 1994)
USA	Dairy calves	Faecal samples from rectum	Spring – early autumn, calves less than 4 months of age	10 g	Yes	No	No	7.1% (farm)	-	1.8% (10 of 560 calves)	(Faith *et al.* 1996)

Country	Cattle type	Sample			Test			Prevalence			Reference
		Type	Time	Amount	Enrichment	PCR	Immuno-magnetic separation	Farm/ pen/ lot	Within farm/ pen/ lot	Animal	
USA	Feedlot	Swab from fresh faecal pat	Autumn, varying times on feed	NA	No	No	No	63% (farm)	0-10% (range)	1.8% (210 of 11,881 cattle)	(Hancock *et al.* 1997)
USA	Feedlot and dairy (6 farms of each)	Swab from fresh faecal pat	Summer - autumn	NA	Yes	No	No	100% (farm)	1.1-6.1% (range)	Beef – 3.6% (38 of 1046 cattle) Dairy – 2.3% (25 of 1097 cattle)	(Hancock *et al.* 1998)
USA	Beef calves	Faecal sample	Autumn, at weaning	10 g	Yes	No	Yes	87% (farm)	1.7-20% (range)	6.9% (61 of 878 calves)	(Laegreid *et al.* 1999)
USA	Beef	Faecal sample from colon	Summer, at evisceration	10 g	Yes	No	Yes	72.4% (lot)	26.2% (mean)	28% (91 of 327 cattle)	(Elder *et al.* 2000)
USA	Feedlot	Faecal sample	Summer, on feed 19-108 days	30 g	Yes	No	Yes	100% (pen)	17.1% (0.7-79.8%) (median, range)	23% (719 of 3162 cattle)	(Smith *et al.* 2001)
USA	Feedlot	Faecal samples from pen floor	Summer, within 36 hours of shipment for slaughter	10 g	Yes	No	Yes	86.7% (pen)	3.3-77.8% (range)	NA	(Dewell *et al.* 2005)

6.2 Appendix 2 – Summary of published efficacy studies with Econiche™ *Escherichia coli* bacterial extract vaccine (Bioniche Life Sciences Inc.)

Cattle	Vaccination protocol	Controls	Challenge	Result	Reference
Calves	Experimental vaccine[16], 2 mL twice by SC injection	Placebo-vaccinated	Artificial with 10^8 CFU *E. coli* O157 2 weeks after second vaccination	Increased serum antibody response to secreted proteins in vaccinated calves compared to placebo-vaccinated controls ($P = 0.0002$). Fewer vaccinated calves shed *E. coli* O157 than placebo-vaccinated calves (5 of 8 compared to 7 of 8) and for less time (1 of 5 for more than 2 days compared to 4 of 7 for more than 4 days). Fewer bacteria secreted by vaccinated than placebo vaccinated calves ($P = 0.05$).	(Potter *et al.* 2004)
Yearlings	Experimental vaccine[17], 2 mL three times (day 0, 21 & 35) by SC injection	Placebo-vaccinated	Artificial with 10^8 CFU *E. coli* O157 2 weeks after third vaccination	Increased serum antibody response to secreted proteins in vaccinated calves compared to placebo-vaccinated controls. Fewer vaccinated calves shed *E. coli* O157 for more than 2 days than placebo-vaccinated calves (2 of 13 compared to 18 of 23) ($P = 0.003$).	(Potter *et al.* 2004)
Steers (192 in 28 pens)	Experimental vaccine[18], 2 mL three times (day 0, 21 & 42) by SC injection	Unvaccinated, separate pens	Natural	No effect on productivity measures. Proportion cattle shedding *E. coli* O157 in vaccinated pens (8.8%) less than in unvaccinated pens (21.3%) ($P = 0.04$).	(Potter *et al.* 2004)

[16] Protein content 100 µg/mL plus different adjuvant
[17] Protein content 25 µg/mL plus different adjuvant
[18] Protein content 25 µg/mL plus different adjuvant

Cattle	Vaccination protocol	Controls	Challenge	Result	Reference
Feedlot cattle (calves and yearlings; 218 pens in 9 feedlots)	Experimental vaccine[19], 2 mL twice (day 0, average day 73-104) by SC injection	Placebo-vaccinated, separate pens	Natural	No significant association ($P > 0.25$) between vaccine and pen prevalence overall or within any one feedlot	(Van Donkersgoed *et al.* 2005)
Feedlot steers (288 in 36 pens)	Econiche™, 2 mL three times at 3 week intervals	Placebo-vaccinated, separate pens	Natural	No effect on productivity measures. No reduction in faecal shedding overall during study[20], although at three pre-slaughter tests prevalence of faecal shedding less in vaccinated pens. Vaccinated cattle 98.3% less likely to be colonized by *E. coli* O157 in terminal rectum cells than placebo-vaccinated cattle (Odds ratio = 0.014, $P < 0.0001$).	(Peterson *et al.* 2007a)
Feedlot steers (608 in 72 pens)	Econiche™, 2 mL either once, twice at a 6 week interval or three times at 3 week intervals	Placebo-vaccinated, within same pen; unvaccinated in separate pens	Natural	No effect on productivity measures. Vaccination reduced faecal shedding of *E.* coli O157 by 68%, 66% or 73% using 1, 2 or 3 dose protocol compared to placebo-vaccinated cattle. Cattle vaccinated with three dose protocol 59% less likely to shed *E. coli* O157 than placebo-vaccinated steers in same pen ($P = 0.015$).	(Peterson *et al.* 2007b)
Feedlot cattle (20,556 cattle in 140 pens in 19 feedlots)	Econiche™, 2 mL twice, once on arrival and once on re-implanting (average 54 days)	Non vaccinated, separate pens	Natural	Reduced shedding of *E. coli* O157 in vaccinated pens of cattle (measured by ROPES) compared to pens of unvaccinated cattle (Odds ratio = 0.59, $P = 0.004$). Shedding of *E. coli* O157 in vaccinated pens not affected by vaccination dose interval (less than or more than 54 days) ($P = 0.85$)	(Smith *et al.* 2008)

[19] Protein content 25 µg/mL, formalin inactive antigen plus Emulsigen adjuvant

[20] Possible contributing factors suggested were major rain event just prior to slaughter resulting in muddy pens and low prevalence in placebo treated steers

Cattle	Vaccination protocol	Controls	Challenge	Result	Reference
Feedlot cattle (718 cattle in 20 pens; subset of the 140 pens above)	Econiche™, 2 mL twice, once on arrival and once on re-implanting (average 54 days)	Non vaccinated, separate pens	Natural	Vaccinated cattle 92% less likely to be colonized by *E. coli* O157 in the terminal rectum mucosa than unvaccinated cattle (Odds ratio = 0.07, $P = 0.0008$).	(Smith *et al.* 2008)
Feedlot steers (480 cattle in 60 pens)	Econiche™, 2 mL twice at an interval of 6 weeks or three times at 3 week intervals	Placebo-vaccinated days 0, 21 & 42, separate pens	Natural	Cattle receiving the three-dose vaccination protocol less likely to shed *E. coli* O157 than placebo-vaccinated cattle (Odds ratio 0.34, $P = 0.002$). Two dose protocol intermediate in effect (Odds ratio = 0.66, $P = 0.20$). Vaccine efficacy (faecal shedding) 65% for three dose protocol and 33% for two dose protocol. Colonization of terminal rectum mucosa cells in placebo-vaccinated group too low to allow statistical analysis.	(Moxley *et al.* 2009)

Cattle	Vaccination protocol	Controls	Challenge	Result	Reference
Feedlot steers (504 cattle in 63 pens)	Econiche™, 2 mL twice, once on arrival and once on re-processing 32 days later	Placebo-vaccinated, either in separate pens or comingled within same pen	Natural	Faecal shedding by vaccinated cattle in vaccine-only pens reduced by 63% compared to placebo-vaccinated pens (Odds ratio = 0.34, *P* = 0.0009). Faecal shedding by vaccinated cattle in comingled pens reduced by 52% compared to placebo-vaccinated cattle in same pens (Odds ratio = 0.48, *P* = 0.014). Hide contamination of vaccinated cattle in vaccine-only pens reduced by 55% compared to placebo-vaccinated pens (Odds ratio = 0.43, *P* = 0.014). Hide contamination of vaccinated cattle in comingled pens not significantly different from placebo-vaccinated cattle in same pens (Odds ratio = 0.67, *P* = 0.33). Colonization of terminal rectum mucosa was not different in vaccinated and placebo-vaccinated cattle (*P* = 0.63).	(Smith *et al.* 2010)

6.3 Appendix 3 – Summary of published efficacy studies with E. coli Bacterial Extract Vaccine (Epitopix LLC.)

Cattle	Vaccination protocol	Controls	Challenge	Result	Reference
Calves (30 in 2 pens)	E. coli Bacterial Extract vaccine, two doses by SC injection at a 3 week interval	Placebo-vaccinated in separate pens	Artificial	Increased anti-SRP serum antibody titres in vaccinated calves compared to placebo-vaccinated controls. Trend to reduced faecal concentration of *E. coli* O157:H7 in vaccinated calves compared to placebo-vaccinated calves ($P = 0.1$). Reduced number of vaccinated calves shed *E. coli* O157 in faeces compared to placebo-vaccinated calves.	(Thornton *et al.* 2009)
60 heifers that tested positive for *E. coli* O157:H7 held in individual animal pens	E. coli Bacterial Extract vaccine, either 2 mL by SC injection twice at a 3 week interval or 3 mL by SC injection twice at 3 week intervals	Placebo-vaccinated, separate pens	Natural	Increased anti-SRP serum antibody titres in vaccinated calves compared to placebo-vaccinated controls ($P < 0.01$). Prevalence of faecal shedding of E. *coli* O157:H7 less in cattle vaccinated with 3 mL than in placebo-vaccinated cattle ($P = 0.02$), vaccinated cattle shed bacteria for less time ($P < 0.05$) and fewer vaccinated cattle were identified as high shedders ($P = 0.02$). Fewer cattle vaccinated with 3 mL had *E. coli* O157:H7 isolated from recto-anal mucosa swabs ($P = 0.04$). Similar trends seen in cattle vaccinated with 2 mL but differences not significant.	(Fox *et al.* 2009)
Yearling steers and heifers (1252 in 20 pens)	E. coli Bacterial Extract vaccine, 2 mL by SC injection twice at a 3 week interval	Placebo-vaccinated in separate pens	Natural	No effect on productivity measures. Prevalence of *E. coli* O157:H7 shedding less in vaccinated cattle than placebo-vaccinated cattle ($P = 0.03$). On day 85 (end of study) likelihood of a vaccinated animal having *E. coli* O157:H7 isolated from their faeces, recto-anal mucosa swabs or hide was less than unvaccinated control ($P = 0.02$).	(Thomson *et al.* 2009)

Cattle	Vaccination protocol	Controls	Challenge	Result	Reference
Yearling steers (1284 in 20 pens)	E. coli Bacterial Extract vaccine, 2 mL by SC injection three times at 3 week intervals	Placebo-vaccinated, separate pens	Natural	No effect on productivity measures. On day 98 (final day of study) vaccinated animals 84.7% less likely to shed *E. coli* O157:H7 in faeces than placebo-vaccinated controls ($P < 0.01$). Concentration of *E. coli* O157:H7 less in faeces of shedding vaccinated animals than shedding placebo-vaccinated animals ($P < 0.01$).	(Thomson *et al.* 2009)

6.4 Appendix 4 – Summary of reported studies of the prevalence of *E. coli* O157:H7 in Australian cattle

Cattle type	Sample			Test			Prevalence			Reference
	Type	Time	Amount	Enrichment	PCR	Immuno-magnetic separation	Farm/ pen/ lot	Within farm/ pen/ lot	Animal	
199 grass- and grain-fed beef, cull dairy cows from eastern Australia[21]	Faecal samples from colon	Post-slaughter, samples collected during summer months	1 g	Yes	Yes	No	-	-	STEC 1.8% cattle and 14% calves; none of cattle isolates and 25% calf isolates O157 serotype	(Desmarchelier 1997)
105 calves in south-east Queensland[22]	Faecal samples[23]	At abattoir, time of year not provided	500 mg	Yes	Yes	No	-	-	STEC 18%; no serotyping	(Sidjabat-Tambunan and Bensink 1997)

[21] Study also included 80 sheep. STEC isolated from 19% sheep faecal samples; of these 6% 0157 serotype.
[22] Study also included 101 sheep. STEC isolated from 69% sheep faecal samples.
[23] Samples frozen prior to analysis.

Cattle type	Sample			Test			Prevalence			Reference
	Type	Time	Amount	Enrichment	PCR	Immuno-magnetic separation	Farm/ pen/ lot	Within farm/ pen/ lot	Animal	
199 mixed age dairy cattle on 3 farms in south-east Queensland	Rectal swab	Cows pre- and post-calving, calves multiple times between birth & 40 weeks of age (588 samples in total)	NA	Yes	Yes	No	67%	2.6-3.4%	Higher in weanlings 1-14 weeks old (5.5%) and heifers (2.8%) than adult cows (1.9%)	(Cobbold and Desmarchelier 2000)
Cull dairy cows from more than 200 dairy herds (505 cattle) in Goulburn Valley of Victoria[24]	Faecal samples from rectum	Post-slaughter samples[25] collected during winter months	10 g	Yes	Yes	Yes but not for isolation[26]	-	-	0.2% (1 of 505 cattle)	(Hallaran and Sumner 2001)

[24] Number of samples collected per herd not provided
[25] Samples stored 14 days prior to analysis
[26] Only samples positive on PCR or Immuno Card STAT underwent immunomagnetic separation

Cattle type	Sample			Test			Prevalence			Reference
	Type	Time	Amount	Enrichment	PCR	Immuno-magnetic separation	Farm/ pen/ lot	Within farm/ pen/ lot	Animal	
Pasture and grain-fed beef and dairy cattle in NSW; 27 pasture beef farms, 23 feedlots, 22 dairy farms[27]	Faecal samples, 25 per farm	Slaughter age, time of year not provided	250 mg	Yes	Yes	No	STEC 85.2% pasture beef farms, 95.7% feedlots, 72.7% dairy farms; 6 isolates O157 serotype (across all farms)	-	0.4% (6 of 1692 cattle)	(Hornitzky *et al.* 2002)
155 grass-fed and 155 feedlot beef cattle from around Australia	Faecal samples from colon	Post-evisceration, samples collected during spring/summer	30 g	Yes	No	Yes	-	-	13% (39 of 310 cattle). Grass-fed beef - 10% (16 of 155 cattle). Feedlot - 15% (23 of 1555 cattle).	(Fegan *et al.* 2004; Anon 2003b)

[27] Similar study in sheep (Djordjevic *et al.* 2001) – STEC flock prevalence 90% in prime lamb flocks and 92% mutton flocks; 2 isolates O157 serotype.

Cattle type	Sample			Test			Prevalence			Reference
	Type	Time	Amount	Enrichment	PCR	Immuno-magnetic separation	Farm/ pen/ lot	Within farm/ pen/ lot	Animal	
50 feedlot, 25 grass and grain fed beef and 25 grass fed beef cattle on 4 farms	Intestinal faecal samples	Post-evisceration, time of year not provided	30 g	Yes	No	Yes	-	-	10% (7 of 68 cattle) samples tested)	(Fegan *et al.* 2005; Anon 2003a)

6.5 Appendix 5 – Summary of reported studies of the prevalence of *E. coli* O157:H7 on Australian beef carcases and in frozen bulk-packed beef

Abattoirs/ freezing plants	Sample			Test			Lot prevalence	Reference
	Carcase	Frozen bulk-packed meat	Time	Enrichment	PCR	Immuno-magnetic separation		
Forty nine abattoirs around Australia, domestic and export over a 12-month period. Sixty freezing plants around Australia[28]	Tissue samples from round, flank & brisket; 180 cm^2 per site	Core samples, 100 g per carton (cartons stored for no longer than 6 months)	Once per quarter, 5 carcases and 5 frozen cartons per abattoir/ plant. Survey conducted during 1993/94.	Yes	No	No	Carcases - domestic 0%; export 0.5%. Frozen beef - domestic 0%; export 0%.	(Vanderlinde *et al.* 1998)
Fifty nine abattoirs around Australia, domestic, export and very small plants (VSP). Thirty boning plants around	Sponge samples from rump, flank and brisket. Three tissue samples for each side of each	Core samples, five or six 100 g per carton (cartons stored for no longer than 6 months)	Winter/spring. Survey conducted during 1998.	Yes	No	Yes	Carcases - domestic 0.1%; export 0%; VSP 0%. Frozen beef - no *E. coli O157* detected.	(Phillips *et al.* 2001a)

[28] Similar study in sheep (Vanderlinde *et al.* 1999) - carcase prevalence domestic <0.7% export <0.3%. Frozen bulk-packed sheep meat domestic 2% export 0.3%.
[29] Similar study in sheep (Phillips *et al.* 2001b) – carcase prevalence domestic 0.3% export 0.4% VSP 1.2%. Frozen bulk-packed sheep meat domestic 1.3% export 0.6%.

Abattoirs/ freezing plants	Sample			Test			Lot prevalence	Reference
	Carcase	Frozen bulk-packed meat	Time	Enrichment	PCR	Immuno-magnetic separation		
Australia[29]	carcase.							
Twenty seven abattoirs around Australia processing grass-fed beef, cull grass-fed dairy cattle and feedlot cattle. Twenty four boning plants around Australia[30]	Sponge samples from butt, flank and brisket regions of each carcase.	Core samples, eight or nine 150 g samples per carton (cartons stored for no longer than 1 month)	Summer/winter. Survey conducted during 2004.	Yes	No	Yes	Carcases – 0.1%. Frozen beef - no *E. coli O157* detected.	(Phillips *et al.* 2006a)

[30] Similar study in sheep (Phillips *et al.* 2006b) – carcase prevalence 0.5%; frozen bulk-packed sheep meat 0.2%.

6.6 Appendix 6 – Reference list

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