final report

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Eye disease in cattle on long-haul voyages

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Executive summary

This research was commissioned after anecdotal reports from veterinarians and exporters working in the cattle live exports supply chain of sporadic, severe outbreaks of eye disease in cohorts of *Bos taurus* cattle destined for long-haul shipping to Russia or China. Outbreaks occurred in pre-export quarantine, on board the vessels or during post-arrival quarantine. Eyes were often so severely affected that it resulted in the loss of the animal. The costs associated with treatment and loss of animals suitable for sale was significant.

Because the reports from industry were inconsistent with respect to time of year, severity, clinical signs and impact, a workshop was held to better define the problem. Veterinarians working in the live export supply chain, export company representatives, makers of immunotherapeutics used to reduce the incidence of eye disease and epidemiologists contributed to the planning of this project. It was concluded that eye disease in these cattle was a multifactorial disease with different possible causative agents and several risk factors. One risk factor over which exporters had some control was the degree to which cattle were immune to disease before receival in pre-export quarantine. It was agreed that an experiment would be conducted to test the hypothesis that providing adequate immunotherapy to cattle in time for full immunity to develop before receival in pre-export quarantine, would greatly reduce the incidence of eye disease in quarantine, on vessels and during post-arrival quarantine. The trial was to be conducted over three voyages.

Sourcing suitable cattle for the experiments proved to be difficult, least of all because gaining access to cattle on-farm, five weeks before transport to pre-export quarantine seemed to be almost impossible. Most exporters didn't buy cattle until much closer to the shipping date. A cohort of Angus cattle destined for China on agistment on a farm in Victoria was deemed suitable for the experiment, although the animals were a bit older than normal. A pathogen survey was conducted before animals were drafted in a Control and Treatment group where the Treatment group received additional vaccination for bovine viral diarrhoea virus, *Moraxella bovis, Manheimia haemolytica* infection and bovine herpes virus 1.2 infection.

Many of the animals had antibodies to BVDV, PI3 and were antigen positive to *Moraxella bovis* and *Moraxella bovoculi* on-farm. In pre-export quarantine and on board vessels there was a zero incidence of eye disease. It was not possible to directly confirm the hypothesis although the degree to which the animals were already immune to causative agents is postulated to be the reason for the low incidence of disease in quarantine.

One problem with this type of research is the difficulty in gaining positive association with treatment when disease outbreaks are rare. To show the effect of treatment (in this case appropriate use of vaccines) there needs to be significant disease in the Control group. This is fundamentally very difficult to predict in field-based research such as this. That and the difficulty in gaining access to cattle on-farm in a timely manner such that full vaccine courses could be delivered meant that the research was revised such that the results of the one experiment, a review of the scientific principles, an understanding of eye disease causation and epidemiology and well-established treatment protocols were used to produce best practice guidelines for the live export industry. The challenges associated with implementing these guidelines are well recognised.

It is suggested that wherever possible and practical, exporters aim to access cattle destined for export at least four weeks before collection at quarantine such that full courses of appropriate vaccines can be given and eye disease outbreaks minimised.

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1 Background

Severe eye disease is a significant problem in *Bos taurus* cattle on long-haul voyages. The economic impact is high owing to the cost of treating affected animals, death of severely affected animals and possibly the loss of business resulting from a lack of confidence by importers in the perceived quality of the product. Additionally, there are significant animal welfare concerns associated with high numbers of cattle suffering from painful eye disease for long periods on boats. This project gathered stakeholders and experts to produce a list of hypotheses to guide a literature review of this problem. Additionally, they advised the best method of data collection to identify causative agents and environmental factors associated with this disease. Samples and data were collected from affected and non-affected cattle on board ships. An experiment was designed and conducted to test whether there was a strong positive association with vaccines, or organisms for which vaccines exist, or if antibiotic metaphylaxis will be of use.

Problem definition

This research arose from discussions with companies that provide cattle for live export, namely Elders International and Landmark Global Exports. Representatives from these organisations along with on-board veterinary surgeons have reported a perceived increase in eye disease in young, *Bos taurus* cattle. While there is literature that documents the aetiology, pathophysiology and treatment of several cattle eye diseases there is a paucity of published information on the occurrence of eye disease in animals on board ships for export.

Serious on-board outbreaks of ocular disease that resemble infectious bovine kerato-conjunctivitis (IBK) on long-haul and extra long-haul voyages are causing market access concerns. Up to 20% of *Bos taurus* cattle have been affected with ocular disease that presents as a more severe syndrome than traditional IBK. Anecdotally, five percent of animals have been recorded as becoming bilaterally blind and approximately 1% suffer perforated globes. The syndrome often occurs despite animals' prior vaccination with Piliguard, with seemingly little effect. There is anecdotal evidence that consignments vaccinated with live IBR vaccine are less, and sometimes significantly less affected. Initial clinical signs are evident approximately 2-3 days into a voyage. These clinical signs are usually a nasal discharge, conjunctival and corneal inflammation and oedema that progress to ulceration and ultimately globe rupture. Animals may recover but the perception by importers is that they are paying for an inferior product.

The syndrome is associated with several significant costs.

These include:

- labour for treating affected animals
- time expended loading and unloading blind animals
- cost of drugs and consumables associated with treating affected animals
- discounting of affected animals by importer
- insurance costs and claims
- death of animals
- perception of poor animal health, husbandry and welfare in live exported cattle that puts the entire industry at risk

It was postulated that several aetiological agents were involved in the syndrome. These include *Moraxella bovis*, Mycoplasma spp., Bovine Parainfluenza Virus 3, Bovine Coronavirus, Bovine Respiratory Syncytial Virus, Bovine Herpes Virus 1.2, Bovine Viral Diarrhoea Virus. The hypothesis was that these organisms, in association with the age and breed of the animals and the environmental conditions on board ships, were acting synergistically to result in a severe ocular disease syndrome that has not been properly documented or investigated.

With a careful review of literature and strategic sampling of specific cohorts of animals, this project aimed to identify the cause and possible mitigation strategies for this syndrome. This will serve to improve the financial return for exporters, significantly improve the welfare and health of cattle on board ships and preserve the live export industry for the future.

2 Project objectives

The objectives of this project were to review current literature and gather epidemiological data from recent outbreaks. They also aimed to identify microorganisms associated with the current syndrome and develop strategies for prevention. The specific project objectives as stated in the contract are:

Problem definition

The problem definition phase of the research consisted of three main outcomes:

1. Review the literature on eye disease in cattle
2. Conduct a strategic survey of susceptible animals in pre-export quarantine to provide evidence of the presence of the suspected causative agents and to better define the problem
3. Identify environmental factors and husbandry practices that are associated with the contribution to, or mitigation of, severe eye disease syndrome in exported cattle

Test the solutions

The experimental phase of this research involved the following experiment:

1. Conduct a pilot experiment that tests the best-practice use of available immunotherapeutics

Report and recommendations

A best-practice guideline document will be produced along with a final report

3 Methodology

Overarching methodology

The contract methodology was modified slightly to include an industry consultation workshop and the experimental phase of the research refined to coincide with the pathogen survey.

The project consisted of a problem definition and identification phase followed by a solution testing phase. The problem definition relied on gathering stakeholders to accurately document the impact, severity and cost of the disease and produce hypotheses on the epidemiology of the disease.

1. Problem definition. The aim of this was to gather information to guide the literature review and data collection phase. Data from the export companies was gathered and a logistical plan developed to facilitate the collection of data in pre-embarkation. Feedback from the industry workshop helped to build a list of hypotheses pertaining to cause and epidemiology of current syndromes and define best options for testing these.

2. Literature review – the review of literature was guided by the hypotheses generated during the workshop.

3. Pre-embarkation sample collection – this took the form of sample collection from affected cattle in quarantine facilities. As far as possible full husbandry history and signalment was collected for all animals to add robustness to the analysis. A veterinary epidemiologist was consulted to assist with experimental design. All samples were submitted to laboratories in Western Australia for analysis. A biometrician was employed to statistically analyse the results. The timing of this sampling was very much dependent on the availability of shipments of cattle.

Concurrently, the hypothesis that improper use of available immunotherapeutics is contributing to the incidence of severe eye disease, was tested.

A consignment to China formed the basis of the experimental cohort. Access was gained to vendors' cattle on-farm in order to:

- draft two-ways by weight on selection by buyers

- allocate weight lines to two treatments – control and treatment groups (aiming for 250 animals per treatment per voyage. Note that securing access to suitable animals on-farm in sufficient time is difficult. Often exporters buy cattle at short notice.)
- control animals have standard China protocol, treatment groups receive a vaccine regime (Pestiguard, Bovilis MH + IBR and Piligard)
- on entry to quarantine, treatment animals receive booster doses of vaccine where appropriate
- treatments are kept together in quarantine
- counts made of incidence and severity of eye disease in quarantine
- swabs (bloods) taken from affected animals for pathogen screening (comprehensive panel.)
- aim to keep animals in treatment groups on ship and continue count but not swabbing on ship (due to the practicalities of this)

4. Report and recommendations – the research was revised to one experiment, a review of the scientific principles, an understanding of eye disease causation and epidemiology and well-established treatment protocols. These were used to produce best practice guidelines for the live export industry.

Detailed methods

Problem definition workshop

In March 2014 MLA/LEP hosted a Problem Definition Workshop at the Tradewinds Hotel in Western Australia. Attendees were:

Sharon Dundon –MLA R&D Manager Live Export Program
Michael Laurence – Murdoch University: Chief Investigator
Ian Robertson – Murdoch University: Epidemiologist
David Kennedy – Independent consultant (MLA)
Tristan Jubb – Animal health consultant
Andre Cirone – RuralCo.: Veterinarian
Lee Taylor – Zoetis: Veterinarian
Tim O'Donnel – Wellard Rural Exports
Richard Leitch – Private consultant
Malcolm Gillett – Coopers: Sales manager
Paul Bloom – Coopers: Field Staff

- Welcome and introduction: Michael and Sharon
- Problem definition: what does the specific eye disease presentation in cattle on long-haul voyages look like? Presentations from Andre, Tim, and Richard followed by open discussion
- Causes? – Lee, Tristan, Andre, Michael
- Research plan intro: Michael
- Vaccine discussion: Malcolm, Jane and Lee
- Measuring the problem: Ian, David, Lee, Andre, Michael and Tristan followed by open discussion
- Experiment/project design: All. What to measure, number of animal, access to animals.
- Close

The discussion in this workshop formed the foundations for the research that followed.

Literature review

A thorough scientific literature was conducted by the CI and is presented in the results section.

Pre-embarkation sample collection and immunotherapeutic trial

One voyage of cattle destined for export to China was tested in 2016.

This experiment was designed to test whether the timely use of vaccines on-farm help to mitigate the incidence of eye disease in young Angus cattle destined for live export to China. Cattle from the same cohort, destined for the same shipment, were chosen. The animals were destined for a quarantine facility in four weeks from the sampling collection.

Study population

The study population in this experiment were 450, nine-month old weaner black Angus cattle. The cattle were destined for live export to China. Experimental cattle were located at an on-farm/ agistment pasture property in rural Victoria. This farm provided backgrounding on pasture four weeks before transport to quarantine station, with the eventual departure to China on the transport boat.

On-farm sampling

Cattle were allocated randomly to a 'Control' or 'Treatment' group. The first group of cattle were allocated as 'Control'. This group were identified with odd-numbered yellow ear tags from 1 to 449. These cattle received the standard China protocol plus breeder protocol (see appendix) on the day facilitated by an Australian Government Accredited Veterinarian (AAV). Further testing at the quarantine facility occurred after arrival in quarantine, conducted by the same AAV.

Cattle were restrained in the head bail before any sampling occurred. Every second, odd-numbered cow had an eye and nose swab taken (i.e. 1, 5, 9, 13, etc). One dry, sterile cotton swab was inserted into both nostrils to collect a mucous sample. Another dry, sterile cotton swab was rubbed around the inside of the conjunctiva and inserted under the third eyelid. These swabs were stored in swab containers filled with 2mL of saline that were appropriately labelled with the ear tag number. Thirty-two cattle were randomly selected within the race to have blood samples collected from the coccygeal vein into a plain vacutainer which didn't contain any anticoagulant. The blood serum was used for assay for of BHV-1 (IBR), BVDV and PI3 virus using ELISA. The on-farm NLIS tags were recorded for each cow that was added into the experiment.

The second group of cattle was allocated as a 'Treatment' group. This group was identified with even numbered ear tags from 2 to 450. These cattle received the standard China protocol plus breeder protocol (see appendix). The cattle in this experimental group received an additional vaccination regime. The regime included Pestiguard (Zoetis) to induce immunity against Bovine Viral Diarrhoea Virus, Bovillis MH + IBR (Coopers Animal Health) to induce immunity against *Mannheimia haemolytica* and Bovine Herpes Virus Type 1, and Piliguard (Coopers Animal Health) to induce immunity against *Moraxella bovis*.

Doses of vaccines were given in accordance with the manufacturers' recommendations. The vaccinations were given subcutaneously in the neck via a multi-dose syringe and vial.

Every second even-numbered tagged cow had an eye swab and nose swab taken (i.e. 2, 6, 10, 14, etc) in the same way as the Control Group.

Monitoring and sample collection: post-on-farm to quarantine to on ship

The 'China protocol' requires all cattle to have blood tests taken on-farm at least 30 days before entering the quarantine facility. The blood is taken by a live export veterinarian. It is sent away to be tested for Bluetongue, Paratuberculosis, Akabane Virus, IBR, BVDV and Enzootic bovine leucosis. Only the ones that come back as negative are sent to the quarantine facility. Upon arrival into the

quarantine facility, all cattle must receive a killed vaccination against IBR. Once in quarantine the cattle undergo another blood sampling within 10 days before export. The cattle were tested for Bluetongue, Paratuberculosis, Akabane virus, BVDV, enzootic bovine leucosis and enzootic haemorrhagic disease.

During the four weeks on-farm after the initial testing, the two groups of cattle were monitored for any signs of eye disease. Affected animals were noted using a monitoring sheet (see appendix). After four weeks, cattle were transported by truck to a quarantine facility.

The animals in this experiment were kept in their respective 'Control' and 'Treatment' groups in the quarantine feedlot. Booster vaccines were given where appropriate to the treatment group. The China protocol was followed for the control and treatment group.

On the ship, the cattle were kept together in their respective treatment groups. The on-board veterinarians recorded the number of animals that developed disease on recording sheets provided. Once in China, additional monitoring of the animals in quarantine for eye disease by the vets was recorded. All the information collected from each of these checkpoints was sent back to Murdoch University, Perth WA Australia.

Table 1 defines the experimental grouping of the animals in this experiment.

Sample Analysis

Blood samples were sent to the Department of Primary Industries and Regional Development Western Australia located in South Perth, WA 6151 for antibody ELISA analysis for BHV-1, Pestivirus (BVDV) and Para-influenza virus 3.

Eye and nasal swabs were submitted to the Anti-Microbial Resistance and Infectious Disease Laboratory at Murdoch University for PRC analysis of BHV-1, BPI3, BRSV, BVDV, *Moraxella bovis* and *Moraxella bovoculi*.

DNA and RNA were extracted from ocular and nasal swabs using Magmax 96 viral RNA extraction kit as per the manufacturer's instructions. Real-time PCR reactions using previously published primer and probe sets were performed using AgPath ID reaction mix (ambion) on Quantstudio 6 flex platform (Life Technologies). Sample curves which crossed the threshold prior to 40 cycles and which displayed characteristic sigmoidal curves were classed as positive for the agent being tested.

Table 1. Experimental design: Immunotherapeutics trial

Experiment one – Eye disease investigation		
Team	**Even numbers – 250**	**Odd numbers – 250**
	On-farm	
G. Smith M. Laurence	Sero-survey (bloods) Eye swabs Standard China Protocol	Sero-survey (bloods) Eye swabs Standard China Protocol Pestiguard Rhinoguard Bovilis MH + IBR Piliguard
	On arrival in quarantine	
G. Smith M. Laurence Quarantine facility staff	Sero-survey and swabs Standard China Protocol	Sero-survey and swabs of affected animals only Booster vaccines as required Standard China Protocol
	On ship and post-arrival	
On-board stockman/vets	Recording of tags of affected animals and severity and type of disease	Recording of tags of affected animals and severity and type of disease

Best practice guidelines

Best practice guidelines were written by the CI and included in the Discussion section below.

4 Results

Problem definition

Problem definition workshop

The discussion during the workshop mirrored anecdotal reports from the live export industry, namely that young, *Bos taurus* cattle destined for long-haul shipments were occasionally developing severe eye disease that wasn't clinically entirely consistent with infectious bovine keratoconjunctivitis (IBK). Industry representatives described variously a syndrome that began in pre-export quarantine and progressed to severe disease during the voyage or post-arrival in the destination country, a disease confined to pre-export quarantine or a disease that manifested on post departure, either on board or post-arrival in the destination country. Symptoms included low-grade upper respiratory disease and conjunctivitis; classic IBK signs of a central ulcer and worsening corneal deterioration as well as sudden onset blindness through corneal oedema.

The reports from industry remain anecdotal. There was no consistency in opinions about time of year, presenting clinical signs or response to preventative measures taken such as minimising dust, reducing fly numbers or managing the mixing of cattle. The most consistent variable in the reporting was that the signalment of the affected cattle was the most significant factor. Cattle were young, recently weaned, *Bos taurus* (usually Angus) animals and eye disease on long-haul voyages seemed to be worse.

Opinion on the use of vaccine was mixed with some suggesting that Piliguard was effective in reducing the incidence of disease while others suggested it made no difference.

The discussion about what the causative agents were yielded no single opinion; rather collectively it was agreed that microbiological agents other than simply *Moraxella bovis* were likely associated with an *eye disease syndrome*. These were likely to be a combination of two or more of Pestivirus, Herpes virus, *Moraxella bovis* and *Moraxella bovoculi*. It was suggested that primary respiratory viruses act as immunosuppressing agents and facilitate damage to the cornea through the inflammatory process associated with the disease. This provided an opportunity for agents of IBK to colonise and cause the more serious eye disease clinical signs.

It was agreed that young *Bos taurus* animals were more severely affected owing to their stress levels and therefore already compromised immune systems. Selection of cattle at short notice followed by immediate shipping and mixing in quarantine was also hypothesised to make eye disease worse.

It was agreed that susceptible animals would be surveyed for pathogens and that the use of a suite of appropriately administered immunotherapeutics may solve some of the disease problems. Vaccines needed to be given time to work before mixing in pre-export quarantine which meant access to the cohorts of cattle at least five weeks before receival in quarantine such that both doses of vaccine could be administered, should this be a requirement for effective vaccine use.

An experiment was planned to test a control and a "best practice" treatment in a real-life situation across three voyages.

Literature review

Introduction

Eye disease is a common problem for producers and exporters of beef cattle, particularly with young *Bos taurus* animals. The problem is associated with significant loss of production and has its impact at both the farm level and during the supply chain in the export process (Sackett et al., 2006). Importers of cattle, particularly the Russians and the Chinese, are reluctant to accept animals with diseased eyes.

Eye disease is a truly multifactorial disease. It is associated with the age, immune status, species and level of stress of the cattle; environmental risk factors including the dustiness of the environment and feed and the presence of flies; and the presence of microbiological pathogens (Henson and Grumbles, 1960; Hughes and Pugh Jr, 1970; Wilcox, 1970; Frisch, 1975; Baptista, 1979; Gerhardt et al., 1982; Barber et al., 1986; Brown et al., 1998; Divers and Peek, 2008; Aiello, 2010; Alexander, 2010; Angelos, 2015). Outbreaks occur when these factors combine to provide for optimum conditions for disease spread and progression.

Control of eye disease relies on managing all three broad aetiological categories. An awareness of the susceptibility of certain breeds and animal of certain ages, modification of environments to minimise risk factors like dust and flies, and provision of appropriate immunotherapy to provide protection against microbiological agents, are all strategies that underpin a good control programme (Steve and Lilly, 1965; Angelos et al., 2007a; Parkinson et al., 2010; Williams, 2010; Angelos, 2015). However, the capacity to manage eye disease in the live export supply chain remains problematic. This review explored the nature of the live export industry, the extent of the problem, the postulated cause of eye disease in this system, and the hypothesised control mechanisms available to producers.

Eye disease in live export

Severe eye disease has been reported as a significant problem in *Bos taurus* cattle on long-haul voyages (Exporters, 2014). The economic impact is high owing to the cost of treating affected animals, death of severely affected animals and the loss of business resulting from a lack of confidence by importers in the perceived quality of the product. Additionally, there are significant animal welfare concerns associated with high numbers of cattle suffering from painful eye disease for long periods on boats.

Exporters of live cattle have limited capacity to choose resistant animals and modifying the environment to minimise the prevalence of eye disease remains particularly difficult in the live export industry. Animal are almost always young, recently weaned, mixed in quarantine and exposed to a significant number of pathogens. Furthermore, quarantine facilities are often dry, dusty environments where stocking rates are high.

Rejection rates, incidence in quarantine feedlots, and actual costs associated with treatment of disease remain carefully guarded, commercial in-confidence data. However, a contact of the Chief Investigator for this project claimed the cost of antimicrobials therapy associated with eye disease on a recent long-haul voyage was in the order of $130,000. Discussions with exporting companies have largely concurred that eye disease is both costly and a significant risk to good animal welfare (Exporters, 2014), a conclusion supported in the literature (Williams, 2010).

Clinical presentation of eye diseases

A diseased eye will present with some standard clinical signs that may or may not all be present. These include conjunctivitis, epiphora, blepheropasm, scleritis, ulceration, neo-vascularisation and blindness (Parkinson et al., 2010).

The most common ocular disease affecting cattle worldwide is Infectious Bovine Keratoconjunctivitis (IBK) or “Pinkeye” (Divers and Peek, 2008; Aiello, 2010). The primary differentials include Infectious Bovine Rhinotracheitis (IBR), caused by Bovine Herpesvirus 1.2, and foreign bodies (Aiello, 2010). A clinical presentation of severe conjunctivitis is the predominant feature of all three differentials. Foreign bodies generally affect a single globe on a single animal whereas IBR can result in a conjunctivitis outbreak like IBK; however, the major differentiating feature on clinical presentation is that corneal ulceration does not occur with IBR. Furthermore, animals affected with IBR may have increased temperatures and the presence of nasal plaques.

IBK is a disease predominately affecting the cornea. It typically spreads rapidly and may affect one or both eyes. Clinical signs progress through several stages. Initial signs include lacrimation, ephiphora, blepharospasm, photophobia, conjunctivitis keratitis and corneal oedema (Aiello, 2010; Alexander, 2010). Within 1-3 days a centrally located ulcer may develop accompanied by an intensifying radiating corneal opacity (Divers and Peek, 2008; Aiello, 2010). The opacity can extend over the entirety of the cornea, blinding the animal (Divers and Peek, 2008; Aiello, 2010). Deep corneal vascularisation extends from the limbus to the edge of the ulcer. At this stage the eye is extremely painful resulting in reduced appetite thus production loss (Divers and Peek, 2008).

The clinical presentation associated with other infectious disease differs slightly in that animals that are affected IBR tend not to show central ulceration of the cornea in the early stages but also have upper respiratory signs including conjunctivitis, epiphora, rhinitis and mild cough (Parkinson et al., 2010).

Cause of eye disease

This section addresses the two main syndromes associated with eye disease in cattle: IBK and IBR.

Infectious bovine keratoconjunctivitis.

Infectious bovine keratoconjunctivitis is a multifactorial disease. Its onset depends on a combination of the presence of particular pathogens, specific environmental conditions and is associated with a particular signalment in cattle (Webber and Selby, 1981).

Pathogens

The flora of the normal bovine eye includes non-haemolytic *Moraxella bovis, Branhamella catarrhalis* and *Moraxella bovoculi* (Barber et al., 1986). The accepted principle agent causing IBK is the gram-negative coccobacillus *Moraxella bovis* (Angelos et al., 2007a). However, other organisms have been isolated from eyes with IBK lesions and proposed as causative agents of IBK (Spradbrow, 1967; Wilcox, 1970; Dueger et al., 2004; Angelos et al., 2007a).

An Australian study investigated 25 herd outbreaks of IBK. *Neiserria spp*. was isolated not only as the predominant organism in 24/25 outbreaks but was also cultured in the absence of Severe eye disease and may sometimes be a significant problem in *Bos taurus* cattle on long-haul voyages.

Moraxella bovis was isolated in only two of the 25 outbreaks (Spradbrow, 1967). Wilcox (1970) demonstrated there was a higher incidence of *Moraxella bovis* and *Neisseria (Branhamella) catarrhalis* in active IBK lesions when compared to the normal flora of the eye. Wilcox further

reported that the incidence of *Neisseria catarrhalis* was more consistently elevated and isolated in 45% of cases when compared to *Moraxella bovis* isolated in only 28% of cases.

In 2002, a Californian experiment investigating antibiotic efficacy for IBK treatment reported lower isolations of *Moraxella bovis* (29/138 ulcers) in comparison to a haemolytic gram-negative cocci 68/138 (Dueger et al., 2004). Through biochemical and molecular characterisation this organism has been classified as *Moraxella bovoculi* (Angelos et al., 2007b; Angelos, 2010). Angelos (2010) further speculates that this species has likely been circulating in cattle populations for years and in previous experiments may have been mis-classified as *Moraxella ovis*, *Moraxella bovis* -like, *B.ovis* or *B.ovis*-like organisms.

Rosenbusch and Ostle (1986) demonstrated experimentally that prior infection of *Moraxella bovoculi* enhances and prolongs colonisation of *Moraxella bovis* and *Moraxella ovis.* This study raised the question as to whether control programmes should include vaccination against *Moraxella bovoculi*.

Moraxella bovoculi and *Mycoplasma bovis* were reported from IBK affected calves in Israel (Levisohn et al., 2004). The calves initially presented with respiratory disease and tested positive for Bovine Respiratory Syncytial Virus (BRSV). Thirty out of the 40 affected calves subsequently developed ocular inflammation and corneal opacity which was diagnosed as IBK. Five of these affected calves were swabbed and cultured that isolated mycoplasma spp. in the absence of any Moraxella spp. or other non-mycoplasma agents. The author postulated that the mycoplasma may have a role to play in the aetiology of IBK.

Gould et al (2013) refuted the association of *Moraxella bovoculi* with IBK. Using a corneal scarification model 31 calves enrolled in the study had the surface of one cornea mechanically damaged using a wire brush. The scarified eyes were inoculated with *Moraxella bovis*, *Moraxella bovoculi* or a sterile control. Corneal ulcerations consistent with IBK were observed in 90% of eyes inoculated with *Moraxella bovis*, 9% of control calves and 0% of those inoculated with *Moraxella bovoculi*. Gould's infection induction study suggests that *Moraxella bovoculi* is not a causal organism. Gould further implied that previous published papers hypothesising the causal link between *Moraxella bovoculi* and IBK are misleading as they do not follow the appropriate experimental design of a challenge study or cohort study for establishing a causal association.

Other agents, *Moraxella bovoculi*, *Moraxella ovis* and *Neisseria* spp. have been isolated from eyes with IBK lesions however *Moraxella bovis* is the only organism for which Koch's postulates have been fulfilled (Henson and Grumbles, 1960; Spradbrow, 1967; Angelos et al., 2007b).

Pathogenicity of Moraxella bovis

Moraxella bovis exists in a virulent and non-virulent form (Divers and Peek, 2008). Two main properties account for the pathogenicity of the virulent form; type IV pili (TFP) and a haemolytic cytotoxin (Prieto et al., 2013). Other pathogenic factors such as surface-associated outer membranes proteins, phospholipases, fibrinolysins and proteases also play a role in virulence that McConnell and House (2005) suggest could provide a role for further vaccine development.

Pili are finger-like appendages on the surface of the bacterium that attach to specific sites on the bovine corneal epithelium. Only the piliated strains can cause ocular infection. Pili antigens have been classified into seven different serogroups (A to G) (Moore and Lepper, 1991). Regarding vaccine development, it is important that the appropriate regional serogroups are included to protect against most *Moraxella bovis* strains encountered in the field.

The haemolytic strains of *Moraxella bovis* produce an exotoxin (cytolysin) which has corneotoxic properties. The non-haemolytic strains are non-pathogenic. There is an ability for phenotypic switching between the haemolytic and non-haemolytic form however the mechanism is unknown.

Prieto et al (2013) postulate that the difficulty inherent in eradicating *Moraxella bovis* is due to the ability of the organism to form a biofilm on the cornea and/or the surface of the nasal cavity. A biofilm is a structured assemblage of unicellular cells which when bonded together form a community of cells that attaches to a solid surface. This structure enables the microorganism to be more persistent through multiple resistance mechanisms. Primarily the structure of the matrix makes it more difficult for antimicrobials to penetrate. Secondarily the individual bacteria in the biofilm can undergo physiological change to improve resistance (Mah and O'Toole, 2001). The bacteria slows down its growth and elicits a protective stress response to shield the cell from heat, cold, changes in pH and chemical agents. Prieto et al. (2013) demonstrated experimentally that piliated forms of *Moraxella bovis* are capable of forming biofilms. The pili is the essential component for bacterial attachment thus biofilm formation.

Infectious Bovine Rhinotracheitis

Infectious Bovine Rhinotracheitis (IBR) is a respiratory disease of cattle caused by bovine herpes-virus 1.2 (BHV-1). BVH-1 is associated with several clinical syndromes in cattle: respiratory, ocular, reproductive, neurologic and gastrointestinal disease (Aiello, 2010). Infectious Bovine Rhinotracheitis is the most common form of the disease and causes a respiratory disease and/or ocular disease (Rebhuns). IBR is widely distributed through cattle populations worldwide and owing

to its rapidly infectious nature is seen in high incidence in concentrated groups of cattle such as feedlots.

BVH-1 is an alphaherpesvirus. Historically the virus has been differentiated into three DNA variant subtypes. Subtype 1 has been classified as causing respiratory infections, subtype 2 respiratory and genital infections and subtype 3 neuropathogenic infections. However, any of the subtypes can produce all forms of clinical disease. In clinical outbreaks IBR morbidity can approach 100% though mortality tends to be low, less than 10% (Aiello, 2010). Fatalities don't typically occur with the primary infection but from secondary bacterial or viral invaders such as *M. haemolytica*, Bovine Virus Diarrhoea Virus (BVDV) and Bovine Respiratory Syncytial Virus (BRSV) (Divers and Peek, 2008).

The respiratory form of IBR results in clinical signs including pyrexia, depression, anorexia, coughing, tachypnea, serous to mucopurulent nasal discharge, oral plaques and ulcerations (Nandi et al., 2009; Aiello, 2010). The severity of the disease depends on the presence of secondary bacterial pneumonia. Acute uncomplicated infections generally resolve within 10 days; however, the virus shedding in the carrier state continues for some time. As with all herpes virus the DNA remains in the neurons of the sensory ganglia in a latent disease state. Under suitable conditions the disease can reactivate, multiple then re-excrete the virus from nasal or vaginal mucosa. This latency creates a persistent source of infection amongst herds.

The ocular form of the disease can be unilateral or bilateral. It results in severe conjunctivitis, epiphora, lacrimation, and photophobia. Pathognomonic multifocal white plaques develop in the palpebral conjunctiva, however persist only in the initial few days of the disease. Affected animals have pyrexia, depression and reduced milk yield even in the absence of respiratory disease. In severe cases circumferential corneal oedema develops which may progress to complete corneal opacity and peripheral vascularisation (Divers and Peek, 2008; Aiello, 2010). No corneal ulceration occurs which distinguishes the ocular form of IBR from IBK.

Cattle are the significant source of viral spread. The virus has a restricted host range, typically infecting cattle and water buffalo, and it does not commonly cross the species barrier (Brake & Studdert 1985). While BVH-1 has been isolated in other species and produces a similar disease process it has not been demonstrated that they contribute to viral transmission. The virus is excreted in nasal and vaginal secretions. Excretion rates differ between BVH-1 subtypes. Subtype 1, traditionally referred to as the respiratory form, in peak infection excretes up to 100 times the amount of virus in nasal secretions in comparison to subtype 2. Viral shedding from carrier animals

occurs in lower volumes; however, reactivation of the latent state can occur at any time and in the absence of clinical signs.

BVH-1 has been associated as an important infectious agent in the development of IBK. Pugh and McDonald (1986) proposed BVH-1 as a predisposing factor to IBK. In their experiment one group of calves was inoculated with BVH-1 in which all animals developed conjunctivitis. These calves were then inoculated with *Moraxella bovis*, of which 70% developed IBK lesions. In a second group of calves the initial inoculation was with *Moraxella bovis* then secondarily infected with BVH-1. Only 50% of these animals developed IBK lesions.

Zbrun et al. (2011) conducted a study testing the ability of BVH-1 to predispose animals to IBK. Forty eight pasture fed beef calves were examined over a 6-month period for natural infection of IBK and BVH-1. Once per month all calves were sampled. Blood was taken for BVH-1 antibody detection and all eyes were swabbed for culture. By the end of the study 100% of calves were positive for BVH-1 however only 19% of calves had developed ocular disease consistent with IBK. Of particular interest in the study was the timeline of disease. As the ocular lesions appeared there was a high prevalence of *Moraxella bovis* and very low levels of BVH-1 antibodies. As the *Moraxella bovis* isolates and IBK lesions decreased the prevalence of BVH-1 increased. While Pugh's experimental inoculation study differed in design to Zbrun's natural infection model the prevalence of viral infection did not appear to correlate with development of IBK.

Transmission and risk factors associated with eye disease

The virulence of *Moraxella bovis* is influenced by various host and environmental factors. Host factors include breed, eye pigmentation, age and immune status. Environmental factors include UV light, face fly population, dust, and tall grasses/shrubs/weeds. Cattle are the primary reservoir of *Moraxella bovis*. The organism is located in the eye and nasal cavities of infected animals and asymptomatic carriers act as disease reservoirs in the herd (Parkinson et al., 2010). Damage to the surface of the cornea allows for inoculation of the bacterium. Spread of the bacterium is via direct or indirect contact with nasal and ocular secretions, fomites or via vectors. The infection is highly contagious and spreads rapidly throughout herds (Parkinson et al., 2010).

Musca autumnalis (face fly) is the major mechanical vector. Gerhardt et al. (1982) showed that protecting against face flies markedly reduced clinical cases of IBK as well as reducing the incidence of isolation of haemolytic *Moraxella bovis*. The female face fly acts as an ocular irritant and a mechanical vector. The organism can be carried on the legs of the fly for up to three days potentiating the rapid spread of the disease (Steve and Lilly, 1965).

UV radiation exposure has been correlated with an increased incidence of disease. Ultraviolet light results in epithelial defects allowing colonisation of *Moraxella bovis*. Hughes and Pugh (1970) surveyed outbreaks of IBK over a five year period in a beef herd under natural field environmental conditions. It was observed that the greatest clinical incidence occurred immediately after the annual peak UV radiation period. On culture decreasing numbers of the haemolytic strain of *Moraxella bovis* were isolated in autumn. In this study it was proposed that UV radiation might play a role in reverting the non-haemolytic strain to the haemolytic form hence clinical disease peaking in summer.

Frisch (1975) demonstrated that eye margin pigmentation influences both incidence and severity of IBK. Un-pigmented eye margins are more irritated by actinic rays which leads to a higher incidence of disease (Frisch, 1975). A three-year study demonstrated that infection rate of 96% in un-pigmented animals versus 68% in full eye margin pigmentation. Any physical irritation to the eye such as tall grasses, shrubs and dust increases susceptibility to infection. Potential for irritant and foreign body irritation to the globe is increased when hay is eaten from the middle of a round bale, from overhead feeders or crowded feeders.

Breed variation has been shown to influence susceptibility to IBK (Frisch, 1975). The Hereford-Shorthorn line developed in Australia has a higher incidence compared with *Bos indicus* cattle (Frisch, 1975). Of the purebred and composite breeds Herefords have been found to be the most susceptible to infection at 22.4% versus Simental 7.6%, Charolais 6.5%, Angus 3.7% and Limousin 3.4% (Snowder et al., 2005; Kizilkaya et al., 2013). Investigation of genetic parameters for resistance to IBK has recently demonstrated a low to moderate heritability trait in Australian tropical *Bos taurus* cattle (Ali et al., 2012). With this heritability comes a small to moderate negative correlation to weight. The author suggests that genetically prone IBK cattle may also have a genetic predisposition to gain weight more slowly (Ali et al., 2012). These authors suggested there is sufficient genetic variation for IBK incidence which could be used in selective breeding programs in a multi-trait breeding program. For this goal to progress a greater number of cases need to be recorded on a national level.

Young stock have a higher incidence of IBK as animals develop a level of humoral immunity after contracting the disease (Hughes and Pugh Jr, 1970; Divers and Peek, 2008). The level and duration of immunity is unknown; however, relapse of disease is typically only seen in immunocompromised states.

Stresses of weaning, transport, and commingling has been proposed as a risk factor in disease by decreasing the animals' immune response. Pugh (1986) investigated the carrier state of *Moraxella bovis* and discovered an increased number of isolates from nasal secretions after shipment compared to pre-shipping culture. Pugh (1986) proposed that stress may cause an increase in the number of carrier animals however further investigation is required.

Prevention of eye disease

Prevention of eye disease depends on the ability to manage and minimise the risk factors. Broadly speaking, appropriate immunotherapy, minimising dust, reducing fly numbers, decreasing the exposure to UV light, decreasing stocking rates and mitigating transport stress are the key ways to prevent IBK. Some of these are discussed further.

Moraxella bovis is ubiquitous which makes elimination impossible (Brown et al., 1998). Most classes of antimicrobials offer effective treatment however may fail to eliminate the carrier state, which is why prevention is paramount (McConnel et al., 2007).

Outbreaks of IBK typically occur during the summer months (Postma et al., 2008). This is attributed to a combination of prolonged UV exposure, a rise in the fly population and an increase in environmental irritants such as dust and tall, dry grasses. Therefore, preventative measures are aimed along a two-pronged approach: management practices to reduce the impact of the vector and mechanical irritants and vaccination to enhance host immunity.

The fly population can be controlled using registered insecticides in a spray, pour-on or impregnated ear tag (Parkinson et al., 2010). Pastures should be slashed to minimise contact with long dry stems and seed heads. This not only reduces the mechanical irritant but also the fomites' ability to reside on the grass. Hay should be rolled out not left as a whole bale and other feeder systems should be low and have adequate room to prevent overcrowding. Stress and commingling of young stock should be minimised during dusty, high UV periods.

While it is not often cited as a significant risk factor, it is highly likely that stocking density plays a role as a risk factor for IBK. Animals housed in close quarters such as in feedlots or on board ships are inevitably exposed to the known risk factors: exposure to infectious disease and increased likelihood of spread because of proximity; dust and dusty feed; capacity to damage corneas as they jostle for food; high numbers of flies. It stands to reason that managing stocking density will mitigate the incidence of eye disease to some degree.

Minimising the impact of other infectious agents such as Bovine Pestivirus and IBR which serve to suppress the immune system and cause eye disease respectively, relies partially on the provision of appropriate immune-therapeutics to animals prior to their entry into pre-export quarantine feedlots.

Vaccination

Vaccines to prevent IBK include live, killed, whole cell or subunit vaccines (McConnel and House, 2005). Traditionally vaccines have been formulated with killed whole *Moraxella bovis* cells. More recently stimulating host immunity has focused on subunit vaccines targeting specific surface pili and cytolysin antigens (McConnell & House 2005).

In Australia producers have access to a commercially available, trivalent, killed vaccine – Pliguard-1 (Coopers® Australia). The regimen for providing protection to cattle is a single-dose vaccine that should be given a minimum of three weeks before exposure/likelihood of a pinkeye outbreak.

The effectiveness of vaccination to reduce incidence of IBK is contentious. Hughes and Pugh (1971) demonstrated that intramuscular injection of a viable, virulent strain of *Moraxella bovis* resulted in the production of serum antibodies and a reduction of clinical incidence of IBK lesions in vaccinated stock when eyes were experimentally inoculated with a homologous strain. Protection appeared to be very specific to homologous challenge. No significant level of immunity was observed when vaccinated stock are inoculated with a heterologous strain (Baptista, 1975).

Field trials have not supported the results of the inoculation experiments. Bateman et al. (1986) conducted two field trials to examine the efficacy of commercial piliated, formalin-inactivated, whole-cell *Moraxella bovis* vaccine. Herds examined had previously high rates of clinical disease. There was no difference in rate of disease between vaccinated and unvaccinated stock; however, the vaccinated stock did have a reduction in the severity of lesions and the number of treatments required. Of note was the overall drop in disease observed in one particular group of 105 dairy heifers. The heifers on the property previously had a 20-30% incidence of disease that dropped to 2% during the trial. Of the clinical cases observed there was no significance difference between vaccinated and unvaccinated stock. The author surmised that maybe there was some overall herd immunity from the vaccinated stock; however, studies randomising herds rather than individuals should be carried out to investigate this further.

Burns and O'Connor (2008) published a review of pinkeye vaccine efficiency studies and trials conducted between 1960 and 2005. Their review highlighted the difficulties in determining efficacy and why there are multiple vaccines available with no consensus as to their worth (Burns and O'Connor, 2008). A large proportion of the studies failed to report features such as design and

execution which prevents critical evaluation of quality and validity. Additionally, the ability to evaluate variation of vaccine efficacy was difficult as many studies did not include descriptions of study design, population or vaccination regime such as dose, route, and frequency of administration. Overall 123 trials were evaluated regardless of quality and of these 43% reported a reduction of pinkeye in vaccinated cattle. 15 of these 123 trials reported randomisation and blinding but of this group only three demonstrated protection against IBK incidence. Only seven studies completely fulfilled methodological requirements to allow subset analysis to determine the reason for the variations in vaccine efficacy. This report detailed the requirements for a comprehensive vaccine study; description of study design; location; population characteristics; method of controlling confounding; method of blinding of evaluators to treatment; vaccination regime; challenge parameters (e.g. level of challenge, organism, whether the challenge was homologous or heterogeneous to the vaccine); control type (e.g. non-vaccinated, placebo, or adjuvant); case definition; frequency and duration of assessment; and disease incidence.

Proposed reasons for failure of commercial vaccines to protect against IBK are:

1) the failure to protect against regional serogroups
2) the loss of cellular piliation of *Moraxella bovis* in vaccine production
3) the potential of another causal organism (McConnel and House, 2005; Burns and O'Connor, 2008; Prieto et al., 2013)
4) increase in size of the vaccinated cohorts – as numbers increase so does the likelihood that individuals will succumb to disease. This in turn increases the chance of an outbreak.

In their review of IBK vaccine development, McConnel & House (2005) suggest that there are additional virulence factors, aside from surface pili and cytolysin, which could be investigated for vaccine development. These include proteases, fibrinolysins, phospholipases and surface-associated outer membrane proteins.

Pili are a principal virulence factor in the pathogenesis of IBK. Pili have been typed into seven groups. Protective immunity using pili based vaccines is serogroup specific and must include regional strains. Herd outbreaks of IBK however may contain isolates belonging to multiple serogroups (McConnel and House, 2005). It has been suggested that including pili from all seven serogroups may lead to antigenic competition (McConnel and House, 2005). Angelos et al. (2007a) investigated combining a pilin and cytotoxin subunit vaccine to overcome the issues associated with the homologous pili vaccine specificity. One hundred and one Northern Californian young beef calves aged between 4.7 and 6.8 months were vaccinated with either a recombinant *Moraxella bovis* cytotoxin vaccine, a

recombinant *Moraxella bovis* pilin- *Moraxella bovis* cytotoxin vaccine or a control of immune stimulating complex (ISCOM) adjuvant. Overall there were a lower proportion of corneal ulcerations in the pili-cytoxotin vaccine group as well as reduced overall treatments required for ulcers. The highest level of ulcers was seen in the cytotoxin vaccine group. Angelos proposed that as all calves were running in a single group to standardise risk factor exposure this may have given the control group herd immunity and reduced pathogen exposure to the control group. Of note was the difference in organism cultured from ulcers of the control group versus the vaccinated groups. High concentrations of *Moraxella bovis* were isolated from the control calves whereas high concentrations of *Moraxella bovoculi* were recovered from the vaccinated calves. Angelos et al. (2007a) suggests that despite immunity to an organism, if there are additional ocular pathogens present, these organisms can flourish to produce corneal ulceration and IBK.

Low efficacy of commercial vaccines has been attributed in part to the fragile nature of pili when creating the vaccine in bioreactors (Prieto et al., 2008). The mechanism by which pili loss occurs is not completely known; however, it has been demonstrated that shear forces in the cells environment from stirred bioreactors contribute to loss (Prieto et al., 2008). One percent piliation levels were obtained following agitation in stirred bioreactors. Prieto demonstrated that using a bubble column bioreactor the final piliation level increased to 25%. The addition of small amounts of carboxymethylcellulose (CMC) at 0.10% (wv-1) to the culture medium with the bubble reactor yielded piliation levels of 65% (Prieto et al, 2008).

Incorporation of *Moraxella ovis* and *Moraxella bovoculi* into autogenous vaccines has been suggested as a management tool. Funk et al. (2009) conducted a randomised blind trial investigating autogenous vaccination with *Moraxella ovis* and *Moraxella bovoculi* taken from active IBK cases concluded that it had no effect in reducing the incidence of IBK or have any effect on weaning weights.

Conclusion

Eye disease is a multifactorial disease. A combination of immune status, pathogen load, environmental risk factors and the signalment of the animals contributes to whether or not disease occurs in cohorts of cattle. In the live export supply chain, few factors are easily controlled. The most readily-targeted risk factor is the degree to which animals have suitable immunity against most of the possible causative agents before receival in pre-export quarantine. Testing this hypothesis was a focus of this research.

Solution testing

Pre-embarkation sample collection and immunotherapeutic trial

Pre-treatment sampling revealed that no experimental animals' nasal or eye swabs tested antigen positive on PCR to BHV1.2, Bovine PI3, Bovine Respiratory or Syncytial Virus. Two animals in the control group were positive for BVDV antigen. Positive results are shown in Table 2 except for the inclusion of the negative result for serum BHV1.2 test.

Several experimental animals' nasal or eye swabs tested antigen positive for *Moraxella bovis* and *Moraxella bovoculi* (see table 2) in both Treatment and Control groups.

Several animals had antibodies to BVDV in both Treatment and Control Groups. All animals were antibody positive for PI3. No animals had antibodies to BHV1.2 or BRSV.

Table 2: results of pre-treatment sampling

Testing	**Control**			**Treatment**		
PCR antigen tests or serum antibody	No. pos (total)	% positive	Lower and Upper 95% CI	No. pos (total)	% positive	Lower and Upper 95% CI
Eye swabs positive for *Moraxella bovis* antigen	97 (113)	85.8	78-92	61 (113)	54	44-63
Nose swabs positive for *Moraxella bovis* antigen	78 (113)	69	60-77	101 (113)	89	82-94
Eye swabs positive for *Moraxella bovoculi* antigen	10 (113)	8.9	4-16	17 (113)	15	9-23
Nose swabs pos. for *Moraxella bovoculi* antigen	16 (113)	14.1	21-40	12 (113)	11	6-18
Eye swabs positive for BVDV antigen	2 (113)	1.8	0.2-6	0 (113)	0	0-3.2
Antibody positive for BVDV (bloods)	30 (32)	94	79-99	16 (32)	50	32-68
Antibody positive for PI3 (bloods)	32 (32)	100	89-100	32 (32)	100	89-100
Antibody positive for BVH 1.2 (bloods)	0 (32)	0	0-11	0 (32)	0	0-11

Sixty-four cattle in the experiment were excluded from entering the shipment because they failed one aspect of the China protocol either on-farm or at the quarantine facility. Three were excluded due to health and welfare reasons: lameness, eye injury and ringworm. On-farm testing revealed that 17 cattle tested positive for Akabane virus, six cattle tested positive for Johne's Disease, three cattle tested positive for Bovine Viral Diarrhoea virus, seven cattle tested positive for Infectious Bovine Rhinotracheitis (IBR) and four came back as inconclusive for IBR. In-quarantine testing revealed one animal was antigen positive for Bovine Viral Diarrhoea Virus and another was positive for Akabane Virus.

The China protocol also required reproductive examination before cattle could be shipped. One cow was removed because it was pregnant, two were determined to be non-breeders, four cows were found to be free martens and one was determined to have a reproductive abnormality.

No cattle in either the Treatment or the Control group develop symptoms of eye disease during quarantine or on board the ship for the duration of the journey. Owing to this, it was not possible to determine whether the immunotherapeutics given to the Treatment group reduced the incidence of eye disease.

5 Discussion

Problem definition

Project objective numbers 1 and 2 were to define the problem. This was done in two ways: the holding of an industry workshop and a thorough literature review. Both objectives were completed.

The impetus for this research project came from veterinarians working in the live export supply chain and owners of exporting companies. Reports of severe outbreaks of eye disease were anecdotal and lacked detail. There were inconsistencies among all individuals who discussed eye disease in cattle. For instance, some vets cited conditions that they deemed to be important for predisposing animals to disease, while others focussed on other aspects of the supply chain. Reports on time of year that animals were affected differed, as did opinions on the severity and impact of eye disease outbreaks. Some argued that outbreaks could be catastrophic while others suggested that eye disease was an incidental finding in their shipped cohorts and could be managed relatively easily.

After visiting live export companies, the CI decided a workshop was warranted to develop some consensus as to the nature of the problem and the way to measure the impact and test solutions. This was conducted in 2014.

The industry workshop and review of the scientific literature yielded the following broad conclusions and hypotheses:

The live export supply chain, with respect to cohorts of young *Bos taurus* cattle destined for long-haul voyages, predisposes cattle to eye disease. The animals are young and are often recently weaned. This, along with significant periods of transport and mixing, imposes stress on the animals which decreases their resilience and resistance to disease. Additionally, they are often mixed in saleyards and on induction into quarantine where they are exposed to the host of respiratory pathogens and pathogen associated with eye disease. Smaller groups of cattle are also aggregated into larger mobs which probably increases the chances of immunity breakdown and disease occurrence. The dusty environment, the dusty nature of the feed, the presence of flies in large numbers and the opportunities for mechanical trauma to their eyes owing to the proximity of animals to one another, provides a perfect environment for the initiation and spread of eye diseases.

This review provides the following summarised conclusions and hypotheses:

- that the signalment of the animals entering supply chains predisposes them to eye disease
- that a range of well documented pathogens play a role in the spread and severity of eye disease
- that defining which of these pathogens are specifically prevalent in quarantine feedlots will support the argument for the appropriate use of immunotherapeutics
- that the environment within which the animals are quarantined prior to export provides perfect conditions for eye disease initiation and spread
- that controlling the well-recognised risk factors in the supply chain is a particularly difficult challenge
- that the use immunotherapeutics given prior to receival in quarantine such that protective antibody titres are in evidence at the time of receival will reduce the incidence of eye disease
- that the export industry will benefit from the development of vaccine protocols that can be shown to provide protection against eye disease.

Solution testing

Pre-embarkation sample testing and immunotherapeutics trial

Project objective number 3 was conducted to characterise the pathogen load present in pre-export cohorts of cattle and conduct an immunotherapeutics trial to determine whether the provision of certain vaccines before receival in pre-export quarantine would reduce the incidence of eye disease in cattle. This objective was partially achieved. The aim was to target three shipments but only one was tested. Reasons for this are described below.

The discussion below addresses the findings of the single survey of pathogens in a cohort of animals destined for export and the implications of the results.

As described in the methods, a cohort of 500 young Angus cattle, agisted on a farm in Victoria before transport to pre-export quarantine, was chosen for this experiment. The results show no difference in the incidence of eye disease during pre-export quarantine or on board the vessel between the Control and Treatment groups. The main reason for this was that no eye disease occurred in the phases of transport. The reasons for this are discussed below.

5.1.1.1 Pathogen load

The microbial agents detected in the cattle were those hypothesised to contribute to eye disease, namely BVDV, PI3, *Moraxella bovis*, *Moraxella bovoculi*. Multiple animals had antibodies to the viruses and bacterial antigens were detected on eye and nose swabs, in the absence of any clinical signs. Some animals had healed lesions on their eyes consistent with previous episodes of mild IBK.

The detection of the viral initiators of bovine respiratory disease was expected. Animals contract these infections during mixing events and then seroconvert. The high prevalence of antibodies to BDVD and PI3 suggested several mixing events or prior vaccination to BVDV.

The high prevalence of *Moraxella bovis* seen on the ocular and nasal swabs is unexpected. *Moraxella bovis* was found to have a higher prevalence overall compared to *Moraxella bovoculi* in this study. A previous study of the normal microflora of the bovine eye showed that *Moraxella bovoculi* was 45.3% prevalent, and *Moraxella bovis* was 12.3% (Barber et al., 1986). The results from this study shows a prevalence of 12-15% of *Moraxella bovoculi*, whereas *Moraxella bovis* had a prevalence of 54-79%. Compared to other studies of literature, the results obtained in this study showed significantly higher levels of exposure to *Moraxella bovis.* There could be several reasons for why these high levels were present in the study. Cattle are the only known reservoir of *Moraxella bovis,* and in previous studies, it has been isolated from the conjunctiva and nasal secretions of animals without any signs of infection (Brown et al., 1998). This could indicate the presence of asymptomatic carriers in cattle (Pugh Jr et al., 1980). Furthermore, biofilm formation could play a role in the asymptomatic state and eventual infection of the ocular surface (Prieto et al., 2013). It has been shown that *Moraxella bovis* could produce a biofilm in vitro setting. The postulation is that this biofilm allows establishment of the bacteria in the nasal and ocular passages (Prieto et al., 2013). This colonisation could lay dormant for a long time until risk factors are optimum for the development of IBK within a cattle herd (Prieto et al., 2013). It is possible a high proportion of these cattle experienced an IBK episode that has subsequently allowed for them to heal and become asymptomatic carriers within the herd. Further spread of the disease in the test herd occurred sporadically.

5.1.1.2 Immune status

BDVD and PI3 are viral initiators for bovine respiratory disease and when animals are seroconverting to these agents they are more susceptible to concurrent disease. It should be noted that the high prevalence of antibodies to the viruses suggest seroconversion had occurred in several animals and

any concurrent illness suffered during that phase had resolved by the time the animals were tested for this experiment.

The presence of both antibodies and bacteria suggests a significant degree of prior exposure to these organisms and in many cases the development of sufficient immunity to the agents that the subsequent mixing of cattle in dusty pre-export feedlots did not result in a breakdown of eye integrity and the onset of an eye disease syndrome outbreak. Essentially, that cattle were mostly auto-vaccinated already. There was a significant degree of herd immunity and prior exposure to causative agents on the agistment property, before any extra immunotherapeutics were delivered.

This cohort of cattle, while representative of the type of animals that suffered from eye disease syndrome, was probably not typical of cohorts where eye disease outbreaks occurred, because of their prior immune status. It is likely that a combination of factors contributed to the immune status of the test cattle. Early sourcing of young cattle, mixing in saleyards and exposure to respiratory virus had already occurred in the test animals which probably resulted in them being a more robust cohort of animals than those that are sourced directly from farms, transported soon thereafter to pre-export feedlot and then mixed with other animals in that stressful environment.

It has been shown that the way animals are weaned impacts on their immune system (Lynch et al., 2010). Abrupt weaning and separation from dams, or so-called “truck weaning” is the most harmful of the weaning methodologies with “yard weaning” being the best way to minimise the impact of weaning on the immune system. (Enríquez et al., 2011).

It is postulated that the most vulnerable cohorts of cattle are those that are abruptly weaned and trucked to sale facilities.

5.1.1.3 Sourcing cattle suitable for experiments

The project objectives listed in 5.2.1 were only parted addressed. The main shortcoming was the number of voyages that were tested for pathogens and included in an immunotherapeutic experiment. For a cohort of cattle to be suitable for these two aspects of the research these criteria had to be fulfilled:

- the cattle had to be recently-weaned, Angus animals destined for a long-haul voyage to either Russia or China
- the animals had to be tested and vaccinated with the Treatment protocol in enough time before receival in pre-export quarantine that immunity after vaccination could be allowed to develop. In the case of several vaccines that require two doses, this time was approximately five weeks

- the cattle had to be kept in their cohorts during pre-export quarantine and on board the vessel to record the incidence of eye disease.

Over the course of the four years of the project it became apparent that fulfilling these specific needs was extremely difficult. Market fluctuations and the number of voyages to either China or Russia meant that after the project design there were limited opportunities to have access to animals. On three separate occasions the CI had arrangements in place to test cattle only to have the plans change for one reason or another the day before departure. These frustrations were simply a consequence of the nature of the industry which tends to be reactive to the needs of buyers and availability of cattle.

While export companies were on the whole willing to support this research, it was virtually impossible for them to facilitate access to test cattle five weeks before receival in quarantine. It was more likely that animals were sourced from suppliers' farms much closer to transport to quarantine, meaning that it wasn't possible to complete the full vaccination course required by the Treatment protocol. Because of the uncertain nature of the live export industry, collection of suitable animals usually only happened close to departure.

Occasionally companies "stockpile" suitable cattle on agistment properties so that they can fill quotas at relatively short notice, as was the case with the test animals describe in this report. The problem with this was that the animals were inevitably slightly older, had already been mixed with other animals from different farms and would therefore likely have more robust immune systems having already seroconverted to many of the pathogens associated with eye disease. The results of the experiment described here support this postulation, given the zero incidence of eye disease after receival in pre-export quarantine.

Field treatment trials and rare diseases

In their book describing field trial of health interventions Smith et al. (2015) suggested that *in general, the trial population should be chosen to represent the group that would be the target for the intervention in a potential future health programme, if the intervention is found to be effective within the trial. Care should be taken to define the target population*. This was done as per the methodology above but sourcing animals that fitted the criteria wasn't easy.

Additionally, there are significant challenges associated with field trials of treatment regimes when the clinical manifestation of the disease occurs in unpredictable outbreaks. To test the efficacy of a

treatment protocol in mitigating or reducing the incidence of a disease in a Treatment group, there needs to be disease manifestation in a Control group. Without the onset of disease in a Control group there is no source of comparison of incidence and prevalence in a Treatment group. In the current experiment, because there was no development of disease in the Control group, it was impossible to measure any statistical difference between treated cattle and those that went through the live export supply chain without additional immunotherapeutics.

This conundrum has been described before. In research into the efficacy of a Cholera vaccine in Bangladesh it was noted that the poorly predictable, multifocal occurrence of disease significantly reduced the availability of test subjects and the capacity to draw conclusions about the efficacy of the vaccines (Clemens et al., 1993). Others, when testing the efficacy of an Ebola virus vaccine note specifically that *The very low Ebola virus disease incidence … means any individually randomised controlled trial implemented there is unlikely to be successful, unless there is a substantial increase in the number of cases* (Camacho et al., 2015). Recognising the significant difficulties associated with conducting field trials of rare disease, others have recommended that trial design should always maximise the chance of producing a positive result by choosing subjects with the highest likelihood of suffering disease (Rothman et al., 1998). In this case, every attempt was made to fulfil this criterion for experimental design but despite this, disease incidence was still zero.

5.1.1.4 Conclusion

The hypothesis that adequate immunity to microbial agents known to contribute to eye disease will reduce the incidence of eye disease syndrome in pre-export quarantine and on board ships is partially supported by the results of this research. In the field trial, the Control group had significant prior exposure to several pathogens and had already developed antibody protection to many of the causative agents such that by the time they reached quarantine they were immunologically robust animals and suffered no eye disease. Proving a direct effect of the proposed vaccine protocol was not possible owing to the difficulty associated with sourcing susceptible cattle in sufficient time to allow immunity through vaccination to develop.

Best practice guidelines

Project objective number 4 was to produce best practice guidelines for industry. These are included here.

It is important to note that the results of the experiment described in 5.2.1 do not provide direct evidence for these best practice guidelines. Rather, the conclusions with respect to the prevention of eye disease in cattle destined for long-haul voyages are based on first principles of the disease epidemiology, the scientific literature, a knowledge of how best to use immunotherapeutics, and an understanding of the stressful nature of the live export supply chain that would also predispose animals to outbreaks of disease.

Best practice management of eye disease in the live export supply chain

5.1.1.5 Introduction

Eye disease in cattle is a multifactorial disease. Most producers consider eye disease in cattle to be associated with pinkeye in cattle, or Infectious Bovine Keratoconjuntivitis. It has been shown that this is only true in part because there is other diseases, especially in the live export supply chain, that affect cattle eyes. These include *bovine viral diarrhoea virus* infection, *bovine parainfluenza 3*, infectious bovine rhinotracheitis caused by *bovine herpes virus 1.2* and *bovine respiratory syncytial virus*. These “viral initiators” cause conjunctivitis and damage to the cornea (the transparent surface of the eye) and predispose the animal to infection with the bacteria that cause pinkeye. In the live export supply chain, these diseases should be considered when trying to minimise eye disease.

5.1.1.6 Risk factors

Eye disease is made worse when:

- cattle are young and stressed – weaning methodologies, time in transport, mixing in saleyards and introduction to intensive systems like feedlots contribute to stress
- *Bos taurus* animals, particularly Angus cattle, make up the cohort of animals in question
- animals have never been exposed to disease and have not had vaccinations to prevent disease
- conditions are dry and dusty – this causes micro-abrasions on the eyes which lead to secondary bacterial infections
- flies are abundant – flies spread the bacteria that cause eye disease
- feed is too finely refined and is dusty

- hay is fed without being rolled out, such that animals push their faces into bales and damage their eyes through scratches and because of grass seeds lodging in eyes
- cattle lack immunity – immunity to microbes that cause eye disease is obtained through either exposure to the microbes or via vaccination. Lack of immunity happens if cattle are unexposed and unvaccinated and made worse if they are stressed
- cohorts of cattle are mixed in confined spaces – this predisposes to stress and the spread of microbes.

5.1.1.7 Clinical signs

The clinical signs of eye disease include:

- conjunctivitis – redness of the soft tissue around the eyes
- blepharospasm – blinking and squinting
- epiphora – over-production of tears that run down the face and leave a crusty residue
- corneal oedema and opacity – the white cloudy appearance to the surface of the eye
- central ulcer – a pinpoint to 10mm hole in centre of the eye
- runny nose associated with respiratory viruses
- cough associated with respiratory viruses
- globe rupture – burst eye, in severe cases.

5.1.1.8 Minimising eye disease in the live export chain

The following are recommendations that will reduce the incidence of eye disease in pre-export quarantine and on vessels at sea

1. Choose cattle that have been yard-weaned to minimise stress

Producers are directed to the MLA-produced resources on minimising the stress of weaning: https://www.mla.com.au/research-and-development/animal-health-welfare-and-biosecurity/husbandry/weaning/ and it is recommended that exporters source cattle from farms where cattle have been yard-weaned

2. Gain access to cattle while still on the farms of origin to provide appropriate immunotherapeutics in enough time (usually five weeks before receival in quarantine) that seroconversion has provided for protective antibody titres at the time of induction to pre-export quarantine. Some vaccines need two doses to be protective against the disease and these can be up to one month apart. One dose of vaccine is not protective and cattle inducted to pre-export quarantine after one dose of a vaccine that requires two doses will not be protected and be susceptible to disease.

It is suggested that appropriate vaccines include those that provide protection against:

- bovine viral diarrhoea virus – (Pestiguard – Zoetis)
- *Moraxella bovis* (Piliguard – Coopers Animal Health)
- *Mannheimia haemolytica* (Bovilis MH)
- Bovine herpes virus – a few products are available. It is acknowledged that the presence of antibodies to BHV1.2 may be contrary to the requirements stipulated in export protocols. The use of live vaccines could be considered once blood testing is complete

3. Source local cattle if possible to minimise truck transport times
4. Adapt cattle to mixed rations slowly in a pasture holding paddock to allow for acclimation to concentrated feed
5. Minimise over refining of feed to reduce dustiness
6. Use insecticidal ear tags or fly traps to minimise fly populations in feedlots
7. Employ dust mitigation strategies in feedlots such as careful choice of bedding and laneway sprinklers to reduce the impact of dust on eyes
8. Try to maintain cohorts of animals in their lines and minimise mixing of cattle groups which facilitates spread of disease
9. Remove and treat affected animals as soon as they are identified because pinkeye is very contagious.

Treatment includes: immediate removal from the population and housing in hospital pens for affected animals; checking for foreign bodies like grass seeds in affected eyes; the use of topical cloxacillin antibiotic ointment in mild cases; long-acting intramuscular antibiotic (oxytetracycline) treatment and anti-inflammatory (meloxicam) treatment for more severe cases; the use of eye patches for severe cases.

6 Conclusions and recommendations

Conclusions

While this research initially aimed to prospectively demonstrate the benefits of furnishing young cattle with suitable immunity such that their risk of developing eye disease in pre-export quarantine was diminished, it became apparent that this was not going to be possible. In the four years allocated to experimental work, on three occasions planned experiments were cancelled just days before field work was to take place. In addition, sourcing suitable cohorts of cattle five weeks before transport to quarantine proved an insurmountable travel. In consultation with the LEP programme manager, representatives from the Live Export Research and Development Advisory Committee and an experienced epidemiologist, it was agreed that resources and money would be best redirected, given the chance of proving the hypothesis was low.

Practical implications for industry

This report includes a best practice guide. Sadly, it is recognised that it is unlikely that industry will adopt these suggestions. This is not a reflection on the willingness or commitment of exporters to improve animal welfare, rather a comment on the nature of the supply chain itself. Decisions are made quickly in a changing environment governed by the whims of buyers and changing market forces. It is highly unlikely that access to cattle on-farm, more than five weeks before receival in quarantine, will be a readily-adopted recommendation, despite this being the best way to provide immune-protection for the animals. Nevertheless, every attempt should be made to reduce the risk factors associated with eye disease, including the strategic use of vaccines.

Future work

It is here argued that the basic principles of controlling eye disease are well understood, as outlined in the literature review. Further experimental work will likely not yield valuable findings. Research should be conducted into the cost of eye disease to the industry. Results of a desktop survey exercise like this that carefully characterises the number and extent of outbreaks of eye disease and the cost to exporters would be useful. Anecdotal reports of serious disease events prompted the development of the project and the true significance of this syndrome is still not well understood. Measuring its actual impact would be a useful exercise.

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8 Appendix

Quarantine and vessel monitoring sheet

Monitoring sheet - eye disease project						
Date	**Tag number**	**Affected eye (L or R or B)**	**Symptoms: P = inflamed eye, U = ulcer, T = tears, P = pus**	**Treated : Y = yes N = no**	**Treatment : describe treatment**	

8.2 China breeder protocol

Australian Government
Department of Agriculture

9 China

Cattle Breeder

PROTOCOL LAST NEGOTIATED

9/04/09

Updated: 27 Aug 2014

PERMIT REQUIREMENTS

Obtainable from:

State General Administration of the People's Republic of China For
Quality Supervision, Inspection and Quarantine (AQSIQ)

9 Madiandonglu
Haidian District

Beijing 100088 CHINA

Each import permit can only allow the importation of one consignment of cattle (unless specified otherwise in the permit).

Updated: 27 Aug 2014

SPECIAL CONDITIONS

1. After having confirmed that the Chinese importer has received the valid import permit for importation of cattle issued by AQSIQ, DAFF may start to implement the quarantine and inspection procedure for cattle according to the requirements presented on this protocol and the import permit. Each import permit can only allow for importation of one consignment of cattle (unless specified otherwise in the permit).

2. AQSIQ shall send veterinarians to the farms of origin, to the related isolation premises and laboratories to co-operate with the Australian veterinarians in conducting the veterinary health certification procedure for the cattle to be exported.

Necessary measures should be taken once the following results have occurred:

1. Positive and inconclusive tested animals should be removed from the herd immediately and

cannot be exported to China.
2. If any positive animals to Bluetongue are confirmed, all the animals in the same farm should not be exported to China. Any inconclusive results should be further confirmed by C- ELISA and either PCR or virus isolation.
3. If animals from a farm are found positive to one of the tested disease except Bluetongue at a rate of more than 50%, all the animals from this farm should not be exported to China.
4. If animals from a farm are found positive to one of the tested disease at a rate of less than 50%, the positive animals should be removed from the herd immediately.

NOTE-while not formally agreed, the Chinese authorities have indicated that livestock will not be considered to be in isolation at the pre-export quarantine premises if there is any other livestock in any part of the registered or approved premises.

Updated: 27 Aug 2014

HEALTH CERTIFICATE

MODEL HEALTH CERTIFICATE FOR BREEDING CATTLE

TO BE IMPORTED INTO THE PEOPLE'S REPUBLIC OF CHINA FROM AUSTRALIA

I, Dr_____, the undersigned, a duly authorized Government Veterinary Officer certify that:

1. All the protocol requirements have been met.

2. The consignment details are:
2.1. Departure date:
2.2. Port of Departure:
2.3. Mode of transport:
2.4. Flight number/Vessel name:

3. Australia is free from foot-and-mouth disease, rinderpest, contagious bovine pleuropneumonia, lumpy skin disease, peste des petits ruminants, vesicular stomatitis, bovine spongiform encephalopathy, bovine tuberculosis and bovine brucellosis.

4. The farms where the exported animals originate from meet the following requirements:
4.1. There have been no clinical cases of tuberculosis, enzootic bovine leucosis, anaplasmosis, paratuberculosis, trichomoniasis, campylobacter fetus, toxoplasmosis, anthrax, mucosal disease (bovine viral diarrhea), infectious bovine rhinotracheitis, bovine ephemeral fever and akabane for the past 1 year.
4.2. The cattle for export were born and raised on the farm from which they are being exported or were reared on the farm during the past 6 months.
4.3. The farms are located in the bluetongue free area. The zoning map for bluetongue in Australia is available on the following website:

http://www.animalhealthaustralia.com.au/namp

5. On the farms where the exported animals originate from, the cattle for export were examined and found to be clinically free of infectious diseases mentioned in clause 3 and 4. The cattle for exportation were kept separate from the cattle not intended for export to China and tested for the following diseases within 30 days prior to entry into the quarantine premise. Only the cattle with negative results for the following diseases were moved to the quarantine premises:
5.1. Bluetongue by C-ELISA.
5.2. Paratuberculosis by ELISA.
5.3. Akabane by Serum Neutralisation test negative at 1:4 dilution OR ELISA.
5.4. Infectious bovine rhinotracheitis by ELISA OR microtitre serum neutralisation test negative at 1:2 dilution.
5.5. Bovine viral diarrhoea by virus isolation test OR AC-ELISA.
5.6. Enzootic bovine leucosis by no enlargement of lymph nodes.
Test results: See attached.
Date(s) of sampling and testing: Name
and Address of laboratories:

6. All the infectious bovine rhinotracheitis seronegative animals were vaccinated for IBR before quarantine period in the quarantine facility with an inactivated vaccine.
Date of vaccination:
Type of vaccine:
Dosage:
Manufacturer:
Expiry date:

7. Prior to export, all the cattle were quarantined for at least 30 days at a quarantine premise approved by DAFF according to Australian regulations. During the quarantine period, the animals were visually examined on a daily basis and found to be free of clinical evidence of infectious diseases listed in clause 3 and 4. All animals were treated and tested as follows with negative results:
7.1. Bluetongue by C-ELISA samples taken within 10 days before exportation
7.2. Paratuberculosis by ELISA test
7.3. Akabane by serum neutralisation test with negative result at 1:4 dilution OR ELISA
7.4. Bovine viral diarrhoea by virus isolation test OR AC-ELISA
7.5. Enzootic bovine leucosis by no enlargement of lymph nodes and AGID test OR ELISA
7.6. Enzootic haemorrhagic disease by AGID test OR ELISA Test
oqwerresults: See attached
Date(s) of sampling and testing: Name
and Address of laboratories:

7.7. All the animals received a booster vaccination for infectious bovine rhinotracheitis at least 14 days after entering the quarantine premises with an inactivated vaccine.
Date of vaccination:
Type of vaccine:
Dosage:
Manufacturer:
Expiry date:

8. During the pre-embarkation quarantine period, the following treatments were conducted

under the supervision of official veterinarians:
8.1. The animals were treated for leptospirosis with long acting tetracycline (20 mg/kg).
8.2. Treatment for external and internal parasites with drugs registered by Australia competent authority:
The name of parasiticide(s): Dose:

Place of use: Quarantine premises

9. Within 24 hours prior to export, all the cattle in the quarantine premise were subjected to clinical examination and found free of evidence of infectious diseases and fit for transport.

10. All crates, vehicles, ships or aircraft to be used for transportation have been cleaned and disinfected with disinfectants registered by Australia's competent authority. The name, dose and place of use of disinfectants: See annex

11. The cattle to be exported have had no contact with animals of other consignments, and have not been transported through areas under quarantine for bovine health reasons.

12. Feed and bedding to be used during quarantine and transportation has been sourced from Australian-origin materials and the feed/bedding is not restricted from sale because of infectious disease of cattle.

ANNEX

A. Disinfectants:
A.1. Place of use: Transport to the Quarantine premises
Name(s) of disinfectant:
Dose:

A.2. Place of use: Transport to the Port
Name(s) of disinfectant:
Dose:

A.3. Place of use: Vessel OR aircraft
Name(s) of disinfectant:
Dose:

A.4. Place of use: Aircrate Crate (if applicable)
Name(s) of disinfectant:
Dose:

Updated: 27 Aug 2014