final report

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Identifying the causes of mortality in cattle exported to the Middle East

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Abstract

This project originated from concerns over mortalities in cattle exported from Australia to the Middle East that had been attributed to respiratory disease.

This project was large, complex, innovative and successful. It has laid the foundation for future field research driven by industry for industry benefit and involving research activities embedded into routine operations. It has generated substantial improvement in our understanding of cattle mortality risk on long haul voyages and particularly mortality due to respiratory disease. It has also contributed substantially to improved resources and systems for monitoring and reporting animal health and welfare outcomes.

The findings from this project will contribute to development of mitigation strategies to reduce respiratory disease risk during export. The success of this project offers lessons for future projects that can be implemented for industry benefit.

Executive summary

This project originated from concerns over mortalities in cattle exported from Australia to the Middle East that had been attributed to respiratory disease.

A key feature of this project was the collaborative and innovative approach to involving exporters and their staff -- particularly shipboard veterinarians -- in the process of collecting and contributing research data and information.

A second key feature of this project is that it has contributed to development of a substantial body of resource information and training material about optimal management of animal health and welfare during export and investigation of morbidity and mortality. This includes the development of training material for training veterinarians and resource material about diagnosing and managing and preventing common conditions of exported livestock. A professional DVD was produced on how to conduct a field necropsy to diagnose cause of death. The project has delivered general improvements to systems and procedures that are being adopted by the industry as part of a general commitment to improvement in animal health and welfare outcomes.

Finally this project has delivered against all of the objectives in the terms of reference and has advanced our understanding of causes of mortality in export cattle.

Twenty voyages were enrolled in the study, comprising a total of 194,216 cattle loaded. There were a total of 742 cattle mortalities during these 20 voyages, producing an overall average voyage mortality percentage of 0.38% (95% CI from 0.36 to 0.41%), while the voyage-specific mortality percentage figures ranged from a low of 0.08% to a high of 1.19%.

The most important cause of mortality in the voyages studied was bovine respiratory disease (BRD), accounting for 50% of deaths that were investigated. This was followed by musculoskeletal and injury-related conditions that were responsible for another 15% of deaths. Other causes of death were responsible for 1 to 6% of mortalities, representing relatively minor contributions to overall mortality counts.

Our findings reinforce the importance of performing gross necropsy examination of dead animals where possible to determine cause of death, while recognising that it is not practical or necessary to perform necropsy examination of every single dead animal.

We found that diagnosis of BRD as a cause of death is very unreliable if based on clinical observation alone and requires gross necropsy to assess pathology in the respiratory organs in particular. In contrast, musculoskeletal conditions and injuries can be effectively diagnosed as a cause of death using ante-mortem observations. This is likely to be because affected animals display obvious clinical signs such as gait abnormalities, reluctance to move or inability to stand, that can be detected readily by general visual inspection of pens.

We have developed systems and resources to provide information on how to conduct a field necropsy and how to diagnose common conditions. These procedures will improve the validity of reports of causes of illness and mortality in exported livestock.

For the first time, advanced molecular diagnostic techniques (quantitative PCR) have been applied to samples collected at pre-export feedlots and during the voyage to identify

respiratory disease pathogens in healthy and diseased cattle and understand their role in the epidemiology of BRD in export cattle.

We detected all major viral and bacterial pathogens of interest in cases of BRD and have made comments on the relative importance of different pathogens under export conditions. Our findings reinforce the view that BRD epidemiology in export cattle is the same disease process as is seen in land based feedlot cattle. Mortality percentages in Australian feedlot cattle are about 0.27% per month on feed (deaths from all causes) and about 0.14% per month on feed for deaths from respiratory disease alone.

Of particular note was the detection of Bovine Coronavirus (BCoV) in samples from cases of BRD and the finding that BCoV may be important in the pathogenesis of BRD in Australian live export cattle. This is the first time that BCoV has been demonstrated in live export cattle and only the second time that BCoV has been reported in Australian cattle.

Different terms and measures have been used to describe mortality in export livestock. Definitions were developed for three mortality measures including two measures that were estimated in export animals for the first time as part of this project.

Voyage mortality percentage was calculated as the cumulative incidence of deaths during the voyage: voyage mortality % = $\frac{100 * Count\ of\ deaths\ during\ voyage}{Total\ count\ of\ cattle\ loaded\ at\ start\ of\ voyage}$.

Voyage mortality percentage is a simple measure used routinely for reporting voyage mortality. If 10,000 cattle were loaded and 15 died during the voyage then the percentage mortality is 0.15% of cattle loaded. Voyage mortality percentage does not incorporate any adjustment for different voyage durations. Voyage mortality percentage is only directly comparable between voyages for those voyages that are of similar duration. Where voyages are of differing durations, the voyage mortality percentage may provide a biased measure of mortality risk. Most historic reports use voyage mortality percentage as the standard metric.

Voyage mortality rate was estimated using Poisson regression that incorporated cattle-days at risk into the estimation process. Voyage mortality rate requires three pieces of data: total cattle loaded, number of deaths and duration of the voyage in days. Cattle days at risk were estimated by multiplying the total animals at risk (number of cattle loaded minus half of the deaths that occurred during the voyage) by voyage duration in days. Voyage mortality rate provides a true incidence rate that is adjusted for voyage duration and can be used to compare voyages of different durations.

Daily mortality rate can only be estimated if data of the count of deaths that occurred on each voyage day is available. Daily mortality rate is also a true incidence rate. This outcome allows appreciation of the variability or dynamic nature of mortality risk from day to day during the course of a voyage.

This study has provided the first detailed descriptions of voyage and daily mortality rates using statistically appropriate methods that incorporate animal-time at risk. Voyage and daily mortality rate estimates produced in this study are reported as deaths per 1,000 cattle days and these estimates are adjusted for voyage duration and therefore can be used to directly compare mortality risk between different voyages and can also be used to compare the relative importance of different drivers of mortality risk.

Our findings indicate that voyage mortality rate has progressively dropped over time and that mortality rates in the most recent period (2010-2012) were generally lower than in previous time periods. This progressive drop is also seen in the more simplistic percentage mortality measure. Mortality expressed as a percentage of cattle loaded per year was 2% or higher in the late 1990s, dropped below 1.5% in the early 2000's and has ranged between 0.1% and 0.15% per year since 2009.

Mortality rate patterns in export cattle are remarkably similar to plots of mortality rate over time derived from land-based, cattle feedlots in Australia which also show a progressive rise over time to a peak at around week four post induction of cattle into the feedlot. The major cause of death in both land-based feedlot cattle and in export cattle on the voyages enrolled in this study was bovine respiratory disease (BRD).

Our findings are consistent with the hypothesis that BRD in export cattle may be driven by pathogen exposure associated with co-mingling around transport to the assembly depot, during assembly depot aggregation of animals prior to export and during the export voyage. A range of other causal factors associated with animals, pathogens, management and the local environment are then likely to influence exposure of animals to pathogens, development of infection and disease and risk of mortality. Factors associated with the shipboard environment may influence subsequent exposure and expression of disease and outcome in sick animals.

Our findings and those from a contemporaneous large-scale study of BRD in Australian feedlot cattle reinforce the importance of BRD risk mitigation strategies that rely on management factors such as reduction in stress, movements and mixing and ensuring animals have access to good quality water and feed. Many of these practices may be incorporated into backgrounding programs and some may already be part of routine management of cattle in feedlots and during export voyages. Vaccination against BRD pathogens has been shown to have significant but small reductions in BRD risk and is considered to be a useful adjunct to other management strategies. Vaccination is not a panacea. It seems likely that management and stress-related factors may be more important in BRD risk mitigation than vaccination status and regardless of whether vaccination is implemented against selected BRD pathogens, it will be important to adopt management strategies aimed at reducing risk as well.

During the course of this project we developed and tested prototypes for a shipboard application to make it easier for AQIS Accredited Veterinarians (AAVs) and stockpersons to collect data required for daily health reports and end of voyage reports. The findings from these activities are useful in fine tuning specifications for development of an application capable of running on smartphones or other hand-held devices for recording animal health and performance data during the voyage.

In summary this project was large, complex, innovative and successful. It has laid the foundation for future field research driven by industry for industry benefit. It has generated substantial improvement in our understanding of cattle mortality risk on long haul voyages and particularly mortality due to respiratory disease. It has also contributed substantially to improved resources and systems for monitoring and reporting animal health and welfare outcomes.

Recommendations

1. *Consider development of a strategic approach to online performance data monitoring systems that are owned by industry and that incorporate measures currently being collected and reported on through the Shipboard Mortality Database (SMDB) and possibly through voyage reporting.*

2. *Consider a follow up project that develops and applies BRD risk mitigation measures including vaccination if appropriate and implements these in a controlled manner to reduce BRD morbidity/mortality.*

3. Consider further investigation into the role of bovine coronavirus (BCoV) in BRD in feedlot and/or export cattle.

4. *Consider a follow up project that develops and applies strategies to mitigate risks for musculoskeletal conditions during voyages.*

5. *Use the findings from this project to contribute to an update of the Veterinary handbook for the live export industry.*

6. *Consider applying the lessons and approaches developed in this project to the sheep export supply chain to monitor impact of strategies aimed at reducing losses from salmonellosis and inanition.*

Acronyms

Abbreviation	Explanation
APS	Australian Position Statement (on the Export of Livestock)
AAV	AQIS accredited veterinarian
AEP	Approved Export Plan
AHA	Animal Health Australia
AHCPLL	Application for Health Certificate and Permission to Leave for Loading
ALEC	Australian Livestock Exporters' Council
ALES	Australian Livestock Export Standards
AMLI	Australian Meat and Livestock Industry Act 1997
AMSA	Australian Maritime Safety Authority
AO	Officer of the Order of Australia
AQIS	Australian Quarantine and Inspection Service
ASEL	Australian Standards for the Export of Livestock
AWC	Animal Welfare Committee
AWI	Australian Wool Innovation
BoCV	Bovine corona virus
BoHV-1	Bovine herpes virus 1
BPIV-3	Bovine parainfluenza virus 3
BRD	Bovine respiratory disease
BRSV	Bovine respiratory syncytial virus
BVDV	Bovine viral diarrhoea virus
CBT	Core body temperature
CI	Confidence interval
CRMP	Consignment Risk Management Plan
DAFF	Department of Agriculture, Fisheries and Forestry
DAFWA	Department of Agriculture and Food Western Australia
DNA	Deoxyribonucleic acid
DPI	Department of Primary Industries
ELISA	Enzyme-linked immunosorbent assay
ESCAS	Exporter Supply Chain Assurance System
FDR	False Discovery Rate
FOB	Free on board
HSRA	Heat Stress Risk Assessment
IATA	International Air Transport Association
IBR	Infectious bovine rhinotracheitis
ID	Identification
iOS	Apple operating system for handheld devices
IR	Incidence rate
IRG	Independent Reference Group
LEAP	Livestock Export Accreditation Program
LEP	Livestock Export Program
LEICC	Livestock Export Industry Consultative Committee
LESAC	Livestock Export Standards Advisory Committee
LESAG	Livestock Export Standards Advisory Group

LHPA	Livestock Health and Pest Authorities
ME	Middle East
MENA	Middle East and North Africa
MLA	Meat and Livestock Australia
MT	Metric tons
NCCAW	National Consultative Committee on Animal Welfare
NE	North East
NLIS	National Livestock Identification System
NOI	Notice of Intention
NPV	Negative predictive value
NSW	New South Wales
NVD	National Vendor Declaration
PACE	Pestivirus antigen capture ELISA
PAT	Pen air turnover
PCR	Polymerase chain reaction
PI	Persistently infected
PIC	Property Identification Code
PIMC	Primary Industries Ministerial Council
PIRSA	Department of Primary Industries and Resources of South Australia
PISC	Primary Industries Standing Committee
PPV	Positive predictive value
QA	Quality Assurance
QAP	Quarantine approved premise
QLD	Queensland
qPCR	quantitative polymerase chain reaction
R&D	Research and Development
RFID	Radio frequency identification device
RLPB	Rural Lands Protection Boards
RP	Registered Premise
RSPCA	Royal Society for the Prevention of Cruelty to Animals
SA	South Australia
SE	South East
Se	Sensitivity
SLEP	Saudi Live Export Program
SMDB	Shipboard mortality database
Sp	Specificity
TWG	Technical Working Group
US	United States of America
VICT	Victoria
VTR	Viral transport media
WA	Western Australia

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1 Project background

The origins for this project began in August 2007 with a workshop held in Brisbane to discuss respiratory disease in cattle being exported from Australia to the Middle East. The purpose of the meeting was to identify information gaps and develop research questions that could be addressed with additional work. There was particular interest in whether vaccination against respiratory disease pathogens should be required for cattle being exported from Australia to the Middle East as a means of reducing morbidity and mortality.

A preliminary assessment of available scientific literature was initiated following that meeting with two deliverables: a literature review of respiratory disease in feedlot and export cattle [1] and a preliminary proposal and budget for a longitudinal study to investigate morbidity and mortality in cattle undergoing live export from southern Australian ports (particularly Fremantle) to the Middle East. The preliminary proposal (B.LIV.0248) was subsequently developed into the project that is being reported on in this report.

The major findings from these activities are summarised here, because these findings were the drivers of much of the design approach adopted in the current study.

1. BRD is a major cause of morbidity and mortality in feedlot cattle. There is very little data derived from rigorous, credible scientific studies describing major causes of death in live export cattle. A single research study on four voyages to the Middle East has identified BRD as an important cause of death in live export cattle. [2]

2. Findings from post mortem examinations of cattle dying from BRD on four long-haul export voyages indicated that pathology is essentially the same as is observed in feedlot BRD cases. Infectious agents and non-infectious risk factors influencing BRD in live export are considered to be the same as in feedlot cattle, with additional factors likely to be operating in export vessels in association with pen design on-board ship, ventilation and local (pen, deck and ship level) conditions during the voyage.

3. Published studies on efficacy of vaccines against BRD in feedlot cattle do not provide clear and unequivocal support for routine vaccination to prevent or minimise the risk of BRD. In some cases vaccination may result in adverse impacts on health and performance outcomes.

4. There was insufficient information currently available on which to determine with confidence that BRD risk is sufficiently high across all cattle that are exported to warrant mandatory vaccination in all exported cattle, or to identify a particular vaccine that should be used, or to expect that vaccination with a particular product would be likely to reduce BRD risk.

5. Implementing a clinical trial to investigate efficacy of BRD vaccines in live export cattle under routine export conditions at the time this report was completed, was considered to be very unlikely to be rewarding. A trial was likely to be very expensive, very difficult to implement, likely to result in collection of biased data, and return

[1] Perkins (2009)
[2] Norris et al. (2003)

equivocal results that would be unlikely to offer value in determining whether to vaccinate live export cattle.

6. There was an identified need to collect credible information describing causes of death in live export cattle. This means using objective, scientific methods that are carefully designed to ensure high levels of validity and reliability.

7. The low expected mortality rates in export cattle under routine conditions on most voyages meant that any project aiming to describe causes of death would need to follow large numbers of voyages in order to collect sufficient data to describe causes of death with confidence. A project that was limited to collection of data only from voyages that were accompanied by project personnel was considered likely to be inefficient, expensive and uninformative largely because of low sample sizes.

The B.LIV.0248 report described a proposed project plan that would produce valid and credible descriptions of the causes of death in cattle exported over long distances from Australia. The plan considered and rejected an approach based on a team of researchers who would conduct post mortems on selected voyages because of the resource inputs (time, cost) and the likelihood that most voyages would have low levels of mortality and therefore contribute little to any study attempting to determine risk factors leading to elevated mortality levels.

Instead a different approach was adopted, involving the development of systems designed for industry to collect valid and credible mortality data in a sustainable manner beyond the completion of this project.

In developing the project plan it was recognised that there was no standardised approach to investigating sick or dead cattle during export voyages and that as a consequence there was potential for variation between veterinarians (and stockpersons) and voyages in how such activities might be performed and how resulting data might be recorded. The proposed project plan was purposefully designed to incorporate standardised approaches for recording observations about animal health and development of resource material and training in application of these approaches. These steps were important in improving the validity and coverage of mortality (and morbidity) data from export vessels and in quality assurance and reporting, as well as providing for improved feedback for veterinarians and stock persons and better job satisfaction.

The proposal then aimed to implement these standardised approaches across a large number of routine commercial voyages, where on-board veterinarians and stockpersons would be encouraged to collect data. This system was intended to be sustainable beyond the life span of the project and would be able to underpin a range of other activities including quality assurance, early identification of problems, general improvement in monitoring and management of animal health and potentially to decisions about the relative importance of various diseases (including BRD) and even to underpin future clinical trials that might test whether vaccination is beneficial and cost-effective.

This proposal was used as the basis for a Terms of Reference document and resulted in the current project (W.LIV.0252) being initiated in May 2009.

2 Objectives for project W.LIV.0252

As a result of the workshops and meetings described above, the following objectives were developed for this project, divided into four stages.

Stage 1: Handbook, training material, assessment of data needs and data collection systems

1. Contribute to the development of a veterinary handbook to complement the existing Stockperson's Handbook, which will contain descriptions for common diseases that occur in live export cattle including diagnosis, management, treatment and prevention. The veterinary handbook will incorporate standardised terminology and procedures for investigating cases of morbidity and mortality including post mortem examination and sample collection to establish the cause(s) of death.

2. Define data requirements necessary to be able to describe mortalities that occur during the live export process and particularly during the voyage (for long-haul voyages). This will include data to describe the vessel, voyage (load date, climatic and sailing conditions, destination, load plan, etc), denominator or line of cattle, and data collected from observations of animals that die (such as: animal identification, date of death, age, sex, breed, condition, clinical signs, any treatments, location within vessel, findings from necropsy examination).

3. Develop systems for collection of data concerning morbidity/mortality and for collection of samples from post-mortem examinations during voyages including importation of samples back into Australia for examination by veterinary pathologists.

4. Review the responsibilities of accredited on-board veterinarians and stockpersons in consultation with exporters and industry stakeholders to ensure the positions are complementary and to incorporate responsibilities associated with objectives 1, 2 and 3.

5. Review training material and training activities for accredited veterinarians and stockpersons to ensure that relevant information from Objectives 1 to 4 are covered.

6. Review existing systems for collection, management and flow of data concerning mortality in export cattle to stakeholders with a view to enhancing or integrating additional capability to allow ongoing flow of data, information and samples for monitoring mortalities during export.

7. Establish a framework for quantifying the opportunity gains associated with strategies and treatments that reduce mortalities and other losses during long haul voyages of cattle.

Stage 2: Pilot study

8. Apply the systems and methods from Objectives 1 to 6 on a limited number of voyages (minimum 2) as a pilot study to test their application and functionality. The pilot study will incorporate input from individuals across all parts of the system (veterinarians, stockpersons, pathologists etc) and from exporters to identify any problems or areas that require further modification.

9. Develop a robust, sustainable system for monitoring of health in export animals, incorporating feedback from industry stakeholders from the pilot study, that is capable of being integrated with routine management procedures and that can provide valid and credible descriptions of causes of death, supported by laboratory examination of samples by veterinary pathologists.

Stage 3: Collection of data on 24 months of voyages

10. Apply the cattle export health monitoring system to two years of voyages.

Stage 4: Extension of project to incorporate farm to feedlot[3]

11. Describe management of cattle in the assembly feedlots and describe patterns of morbidity and mortality in cattle during the feedlot phase.

12. Describe the prevalence of shedding of specific BRD pathogens by cattle in the assembly feedlot and on-farm and investigate factors that may influence pathogen prevalence.

13. Compare findings from assembly feedlot and on-farm investigations with findings from disease investigations performed during export voyages.

3 Methodology

3.1 Stage 1

All of the objectives under Stage 1 were addressed at the same time in the first few months of the project. Initial project team meetings were used to discuss the objectives and develop tasks and activities which were then assigned to various members of the project team. In some cases separate component reports were completed and in other cases information arising from completion of component tasks was fed into other outputs.

The project team met once or twice yearly and there were regular meetings involving project team members and industry stakeholders (primarily AAVs and exporters). Additional communication was conducted through phone calls and emails.

The Veterinary Export Handbook (W.LIV.0252) (Objective 1) was written by the project team and reviewed by a number of veterinarians with live export experience and by additional individuals with veterinary pathology expertise and large animal internal medicine expertise.

During the course of the project the Veterinary Export Handbook (W.LIV.0252) was reviewed and edited on a number of occasions in response to industry feedback.

Data requirements for collecting information on healthy, sick and dead cattle (Objective 2) were developed in discussion between project team members and AAVs and exporters, as part of the work leading to development of the Veterinary Export Handbook (W.LIV.0252). This included the development of template paper forms intended to be provided for AAVs to be filled in during the voyage as a means of collecting standardised information about sick

[3] Stage 4 objectives were added to the project in an extension in late 2011 to include the farm to feedlot phase of live export.

and dead cattle. The forms and their application and use were described in the Veterinary Export Handbook (W.LIV.0252).

A review was conducted of the job descriptions for AAVs (Objective 4) including expectations and obligations placed on their performance by the relevant government regulatory body (AQIS) and by exporters who are responsible for employing the AAVs. This activity included discussions with AAVs and exporters and review of example letters of agreement or contracts between exporters (employers) and veterinarians contracted as AAVs.

A training needs analysis (Objective 5) was conducted to understand the training needs for accredited veterinarians and stockpersons who may be participating in disease investigations on board export voyages as part of this project.

A variety of options were considered for collection and management of data as part of the project (Objective 6) in discussion between project team members and industry stakeholders. This information in turn contributed to the development of the systems and procedures described in the Veterinary Export Handbook (W.LIV.0252).

An agricultural economist member of the project team was engaged to provide advice on approaches to incorporate economic assessments of impacts of mortality and possible benefits of reducing mortality risk to the industry (Objective 7).

3.2 Stage 2 – Pilot voyages

The purpose of pilot voyages was to apply the newly developed procedures and protocols as outlined in the Veterinary Export Handbook (W.LIV.0252) on a small number of commercial voyages as a test of the procedures (Objective 8).

Feedback from all parties concerned would then be sought and used to modify and refine the procedures (Objective 9) and result in a final, agreed set of procedures and protocols that were accepted by all stakeholders and that could then be applied to a larger number of voyages in the main part of the project.

The desired outcome from the pilot voyages was a final protocol that could be embedded into routine commercial voyage operations and that could deliver a minimum set of research data on each voyage into the research project. The resulting systems would then be robust and sustainable and would both meet the needs of the research project and contribute to a broader, long-term and sustainable improvement in the way animal health and welfare was managed during livestock export voyages.

3.3 Stage 3 – Collection of data from 24 months of voyages

Stage 3 of the project involved applying the final procedures and protocols to as many voyages as possible over a two-year period.

For 19 out of 20 voyages, data and sample collection was performed by the Australian Quarantine and Inspection Service (AQIS; now DAFF Biosecurity) Accredited Veterinarian (AAV) accompanying the voyage. A standardized protocol was provided, and all participating AAVs received training in the data and sample collection protocols.

Necropsy equipment, containers for collecting samples, and paper or electronic templates for recording observations were all provided on board each ship. The AAVs accompanying the voyages were encouraged to collect a defined set of data and samples from each animal that died, but this was not always possible due to the demands of other tasks and duties that they are routinely expected to perform during voyages. For 1 out of the 20 voyages, data and sample collection was performed by a member of the research team (SJ Moore).

Standardized data recording forms were used to collect animal and epidemiological data for each animal that died. Data included the animal's location on the ship, animal characteristics (including visual ear tag, electronic identification tag, breed, and weight), clinical signs displayed before death, risk factors or events that may have contributed to death, gross necropsy findings if available, and a preliminary cause of death.

Necropsy samples included fresh tissue samples collected into 10% buffered formalin at a maximum ratio of 1 part tissue to 10 parts formalin, and tissue samples (approximately 5 mm^2) and/or swabs collected into a 5 ml plastic screw top container filled with 2 ml of viral transport media (VTM; Hanks balanced salt solution, penicillin G [1,000 units/ml], streptomycin [25 mg/ml], and amphotericin B [0.1 mg/ml]; Department of Agriculture and Food, Western Australia (DAFWA)). Samples stored in VTM were stored frozen on-board the vessel until they were collected when unloaded at an Australian port.

The number and type of samples collected at necropsy depended on the animal's clinical signs prior to death and gross necropsy findings. These protocols were all defined in the Veterinary Export Handbook (W.LIV.0252). Core samples collected from all animals were: lung (grossly normal and abnormal), trachea, heart, ileocecal junction, kidney, liver, and rumen into 10% buffered formalin, and nasal and lung swabs. When the animal showed clinical signs prior to death which were suggestive of a specific disease and which was confirmed at necropsy, then a range of additional samples were collected according to the suspected disease. When there was no obvious cause of morbidity/mortality and the cause of death could not be determined from gross necropsy findings then the core samples plus fixed skeletal muscle, reticulum, abomasum, small intestine, large intestine, pancreas, mesenteric lymph node, gall bladder, spleen, adrenal gland, and the brainstem and cervical spinal cord were collected into 10% buffered formalin.

Samples and data collection forms remained on the ship until the next time it berthed in Fremantle (Western Australia). At that time a member of the project team boarded the ship and collected all forms and samples, processed them through importation inspection and transported them directly to the quarantine approved premises at the Department of Agriculture and Food, Western Australia, for processing. Care was taken during this process to ensure that VTM samples remained frozen during transit and were held frozen at DAFWA facilities until they were processed and analysed.

3.4 Collection of samples from assembly depots

For four voyages (voyage 5, 8, 17, 18) nasal swabs and serum samples were collected from cattle during the assembly period.

The animal was restrained in a head bail and a 20cm cotton swab was inserted approximately 10 cm into the nasal cavity. The swab was rotated across the nasal mucosa to collect a sample of the nasal secretions. During both insertion and removal care was

taken to prevent the swab being contaminated by dirt and other debris on the nostrils. The swab was immediately placed into a 5mL plastic tube filled with 1-2 mL VTM and kept chilled until transported back to the laboratory.

To investigate whether the prevalence of viral and bacterial shedding changed with time, 2 cohorts of animals were sampled 7 days and 9 days apart. It was not possible to select the same individuals for sampling at the first and second sampling sessions, but animals that were sampled at the second session were selected from pens containing animals that had been sampled at the first session.

Nasal swab samples were processed and nucleic acids from the organisms of interest – bovine coronavirus (BCoV), bovine herpes virus 1 (BoHV-1), bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (BPIV-3), *Histophilus somni*, *Mycoplasma bovis*, *Mannheimia haemolytica*, *Pasteurella multocida* – were detected using quantitative polymerase chain reaction (qPCR) assays as described in the following section.

Serological samples were collected from a subset of 334 animals that were also sampled by nasal swabbing by harvesting a small amount of serum from samples that had been collected as part of health certification requirements for export animals.

Whole blood was collected by tail-vein bleeding by accredited third party veterinarians as part of routine pre-export health checks and serum from these samples then submitted to the Department of Agriculture and Food, Western Australia, for commercial Bluetongue and Bovine leukaemia virus antibody testing. An aliquot of serum was separated from a subset of animals and used for separate disease testing as part of this project.

Collection of serum aliquots for testing as part of this project did not interfere with routine pre-export health tests and the project team had no knowledge of the results of any routine certification testing procedures at the time sampling was completed for this project.

Aliquots of 200 µl of serum were tested for the presence of circulating antibodies to the viruses of interest using commercially available enzyme-linked immunosorbant assay (ELISA) kits according to manufacturer instructions for the following viruses: BoHV-1 (Infectious Bovine Rhinotracheitis (IBR) gB X2 Ab Test, IDEXX, Montpellier, France), BRSV (Bovine Respiratory Syncytial Virus (BRSV) IgG Antibody Test Kit, IDEXX, Montpellier, France), BVDV (Bovine Viral Diarrhoea Virus (BVDV) Antibody Test Kit, IDEXX, Montpellier, France), BPIV-3 (Parainfluenza-3 Virus (PI3) Antibody Test Kit, IDEXX, Montpellier, France).

It was not possible to test for antibodies to BCoV since a validated commercial test was not available.

Results were classified as positive, negative or inconclusive using thresholds defined by the manufacturer for each test kit. Inconclusive samples were not retested, and animals with inconclusive test results were not included in the analyses.

Where possible, the ear tag, RFID, sex, breed and weight of each sampled animal was recorded. Not all data was available for all animals.

3.5 Processing and analysis of samples

Details of the methods for handling, processing and examining biological samples have been described. [4] A brief summary is provided here.

Representative samples were taken from each fixed tissue and processed routinely for embedding in paraffin wax. Histological sections were cut at 5 µm, stained with hematoxylin and eosin, and examined for pathological changes using light microscopy.

Samples in VTM were processed using conventional methods for viral RNA extraction using commercial nucleic acid extraction kits. Quantitative polymerase chain reaction (qPCR) was used to detect nucleic acids from organisms commonly associated with BRD. The qPCR was performed using a commercial kit (QuantiTect virus +ROX vial kit, Qiagen Inc., Valencia, CA) according to the manufacturer's instructions.

Primer and probe sequences were sourced from available sequences for each of the pathogens of interest: BCoV, BoHV-1, BPIV-3, BRSV, BVDV, *Mannheimia haemolytica, Pasteurella multocida*, *Mycoplasma bovis* and *Histophilus somni*.

To reduce the number of tests, required reactions were multiplexed as follows: BCoV and BPIV-3, BRSV and BVDV, *M. haemolytica* and *P. multocida*, *H. somni* and *M. bovis*. Bovine herpesvirus 1 was run as a single assay.

Each run contained duplicate samples of a positive control (either a virus isolate or a clinical extract that had previously been characterized), a negative control (an extract of cell culture–grade fetal bovine serum), and a blank (PCR-grade water). Runs were only considered valid if the positive control was amplified at the expected threshold cycle, and the negative and blank controls showed no amplification.

Positive samples were those with a characteristic sigmoidal curve similar to the positive control, crossing the threshold before 40 cycles. Negative samples were those with no characteristic sigmoidal curve. Samples crossing the threshold after 40 cycles were regarded as suspicious for containing the nucleic acid of interest and were retested before classifying as negative or positive.

3.6 Determining the cause of death

More details can be found in Moore. [5]

The final diagnosis for each animal was based on the combination of all available data and information relating to that animal:

- description of pre-mortem clinical signs and gross necropsy findings and any other relevant information recorded on the dead cattle/necropsy form;
- histology results from tissue samples collected from the animal and stored in formalin;
- qPCR results on samples collected in VTM;
- digital photographs of pre-mortem or gross necropsy findings.

[4] Moore et al. (2014); Moore (2014)
[5] Moore (2014)

Not all data was available for all animals.

Respiratory disease was considered the cause of death if there was cranioventral consolidation of more than 30% of the lung [6] and/or the AAV had assigned a preliminary diagnosis of pneumonia/BRD and there were histological findings of moderate to severe pneumonia in lung tissue.

Heat stroke was considered the cause of death if the animal was noted to be panting heavily prior to death or was found dead with no previous clinical signs, the core body temperature (CBT) was ≥ 43°C (measured using a deep temperature probe), and necropsy findings were consistent with those described previously for heat stroke in live export cattle : eyes sunken, muscles glowing pink and dry rather than red-brown and moist, heart tightly contracted with epicardial ecchymoses, severe acute diffuse pulmonary congestion, and edema causing the lungs and mucosa of the trachea and bronchi to be dark red. Ambient environmental temperatures were not recorded.

Other causes of death were based on review of the findings from all available data on dead animals and assignment of the most plausible cause or causes of death.

3.7 Retrospective data

Retrospective data for sea voyages between January 1995 and December 2012 were obtained from the Shipboard Mortality Database (SMDB) which is funded by Meat & Livestock Australia and administered by the Department of Agriculture and Food, Western Australia. The SMDB provides a repository of information, including livestock mortalities, on every voyage on which sheep, cattle, and/or goats are transported live by sea from Australia. [7] For the purposes of this report only voyages carrying cattle, either as the only species or as a mixed sheep/cattle shipment, were included in the analysis.

Load regions were coded as described in Norris et al. [7] Northern ports were those north of 20° latitude south (Port Hedland, Broome, Wyndham, Darwin, Karumba, Weipa, Mourilyan, Townsville); southern ports are those south of 31° latitude south (Fremantle, Bunbury, Esperance, Thevenard, Adelaide, Portland, Geelong, Devonport, Port Kembla, Sydney, Newcastle); other ports are those located between 20° and 31° latitude south (Geraldton, Denham, Carnarvon, Dampier, Mackay, Gladstone, Brisbane).

Destination regions were coded based on destination port and country location using a similar approach to that described in earlier annual reports. Regions included Middle East and North Africa (MENA: Bahrain, Egypt, Israel, Jordan, Kuwait, Libya, Oman, Pakistan, Qatar, Saudi Arabia, Sudan, United Arab Emirates), South East Asia (SE Asia: Brunei, Indonesia, Malaysia, Philippines, Singapore, Vietnam), North East Asia (NE Asia: China, Japan, Russian ports on the Pacific facing coast of Russia, South Korea), South East Europe (SE Europe: Turkey and Russian ports on the Black Sea), Miscellaneous (East Timor, Mauritius, Mexico, New Caledonia, Russian ports on the west coast near Finland, Samoa, Solomon islands, Sri Lanka).

The primary outcome variable (voyage mortality rate) was based on a numerator measuring the count of deaths that occurred during loading, voyage and discharge for each voyage,

[6] Gagea et al. (2006)
[7] Norris and Norman (2003)

and a denominator representing animal time at risk. Animal time at risk was calculated by multiplying the total cattle loaded by the sum of voyage days plus discharge days.

More recent years of data from the SMDB contained detailed records of mortalities per voyage day for export voyages. These records were used to create a dataset with one row per animal that recorded survival time for each animal. Each row included a variable measuring time at risk for that animal. For animals that survived, time at risk was equal to the length of the voyage in days and for animals that died it was equal to the voyage day when death occurred. These data were used for survival analysis.

3.8 Cattle movement data

NLIS data on animal movement histories was obtained from the NLIS database for Western Australia for those cattle exported on a subset of voyages that had been enrolled in Stage 3 of this project. Extensive data exploration and cleansing was required in order to relate NLIS database records with particular export assembly feedlot codes and with dates of departure for enrolled voyages, because the date recorded in the NLIS database for animal movement may not necessarily be identical with the actual date of movement (there is some leeway allowing users to enter movement data within several days of animal movements).

PIC codes were used to derive property locations by using additional data sources including the public brands database (Department of Agriculture and Food Western Australia, 2014), Google Earth and saleyard postcode locations. All location data was then aggregated to shire levels for analysis and reporting to ensure de-identification. The resulting dataset was then used to infer location (consolidated to the shire level and also to a corresponding climate zone), number of moves and distance travelled for each animal, for the whole of life and for a 90 day period prior to the ship sailing from a port of loading. These movements were viewed using a Geographic Information System to identify general patterns.

3.9 Statistical analyses

Voyage mortality percentage was calculated as the cumulative incidence of deaths during the voyage with a denominator representing the total count of cattle loaded onto the ship at the port(s) of loading, and a numerator representing the count of deaths observed for the entire voyage (from first day of loading to last day of discharge). Voyage mortality percentage is only directly comparable between voyages for those voyages that are of similar duration. Where voyages are of differing durations, the voyage mortality percentage may provide a biased measure of mortality risk.

Voyage mortality rate was estimated using Poisson regression that incorporated cattle-days at risk into the estimation process. Cattle days at risk were estimated by multiplying the total animals at risk (number of cattle loaded minus half of the deaths that occurred during the voyage) by voyage duration in days. The voyage mortality rate provides a true cumulative incidence rate that is adjusted for voyage duration. Voyage mortality rate when estimated in this way assumes that the risk of mortality is the same on each voyage day for the duration of the voyage. It is acknowledged that in fact mortality risk is not likely to be constant over the duration of any voyage and may vary from day to day.

Daily mortality rate can be estimated if data is available of the count of deaths that occurred on each voyage day. This outcome allows appreciation of the variability or dynamic nature of mortality risk from day to day during the course of a voyage.

Descriptive results are presented as summary counts and percentages for the number of animals that died or that returned positive test results for specific pathogen testing or for causes of death. Denominators for percentage estimation were based on numbers of animals in a sampled group or loaded onto a ship as part of a participating voyage.

Poisson and negative binomial regression modelling was used to analyse count data (number of animals that died or that tested positive for defined outcomes) to screen for associations between outcomes and relevant explanatory variables.

Regression models were run as univariable screening models to test for unadjusted associations. In some cases multivariable models were run by adding multiple significant explanatory variables from screening models into one multivariable model to look for adjusted effects (effects of one explanatory variable that are adjusted for other variables in the same model). In many cases it was not possible to use multivariable models because of the sparseness of the data and the presence of confounding between many explanatory variables. In these cases results are presented only for univariable models with appropriate caution in interpretation of findings.

Cattle are typically managed in groups during export (property of origin group, transport, feedlot pen, voyage, ship) leading to statistical clustering. Data from cattle in the same cluster unit (same mob, same ship etc) are likely to be more similar than data from cattle in different clusters. The impact of clustering is to increase risk of correlation between data points and this in turn may lead to biased results generated from statistical models unless appropriate analytic adjustments are made. In this report statistical models were adjusted where possible for clustering either by adding fixed effects or random effects to models to account for clustering and ensure estimates for other effects were unlikely to be biased by clustering in the data.

There were occasions where linear regression models were applied for continuous outcomes (such as core body temperature) and logistic regression or chi-squared tests for binary outcomes (dead/alive, disease positive/negative).

Spearman rank correlation test was used to test for correlations between the presence of various organisms, as detected by qPCR, in the nasal secretions and lung.

A flexible parametric survival model (Royston-Parmar model) was applied in Stata for daily mortality data created from SMDB data. [8] An initial model was fitted that incorporated proportional hazards assumptions (no interaction between destination and time). The approach involved fitting splines using five degrees of freedom and then generating predicted hazards for defined time periods (based on the range of time periods covered by both levels of the destination variable (out to day 35). Model output was used to describe mortality rates per day of voyage (daily mortality rate).

[8] Royston and Lambert (2011)

Descriptive summary tables were prepared in Microsoft Excel® and statistical analyses conducted using Stata (www.stata.com) and the R software environment for statistical computing (www.r-project.org). All analyses applied a significance threshold of alpha=0.05.

4 Results – Stage 1

4.1 General approach

There was extensive consultation and engagement with exporters and AAVs that both preceded the start of the project (to ensure that the approach being put forward in this project was feasible) and that went on during the project as procedures were developed, tested and refined.

The focus was on those exporters and AAVs involved in exporting cattle on long-haul voyages from Fremantle.

The result of ongoing discussions was a paradigm shift in how research might be conducted on-board export voyages. The background and justification to this shift was discussed in detail in the final report for the scoping project (B.LIV.0248) that preceded the initiation of this project. A brief summary of the issue is provided here.

Previous projects investigating causes of mortality during export voyages in sheep (W.LIVE.123) and cattle [9], had involved research team members being placed in export assembly depots and on-board export ships purely for the purposes of investigating sick and dead animals and collecting data and information for the research project. Resource constraints meant that it was only possible to do this for a small number of voyages, four in the case of the previous cattle research project.

At the time the current project was being considered, most long haul cattle export voyages were reporting relatively low mortality rates, and there were sporadic individual voyages that reported mortality rates that exceeded the regulated mortality reporting threshold. If a conventional research project was implemented with research team members placed on a small number of voyages, there was a very high likelihood that this might result in collection of limited data on causes of mortality and that it might not provide any useful information on possible causal factors for major causes of mortality simply because of the limited number of mortality cases.

Two alternative approaches were then considered for the current project. The first involved expanding a conventional research approach to a larger number of voyages by increasing the size of the research team and running the project for a longer period. This approach was considered non-feasible because of costs and because of difficulties in ensuring research personnel for a prolonged period of time to accompany voyages that are often arranged over a relatively short and unpredictable time frame.

The final alternative was to use a different approach based on using existing industry personnel (principally AAVs) as the primary source for recording observations and collecting samples during export voyages. This approach would potentially allow all eligible voyages to

[9] Norris et al. (2003)

be enrolled in the project and would result in much larger sample sizes and more efficient data collection. This approach then formed the basis of the current project.

Our approach was based on enrolling commercial voyages by seeking commitment and participation from exporters and in particular their support for then asking AAVs to collect information and biological samples from dead cattle and information about cattle health in general during the voyage.

The project team would then provide support and resource material to AAVs to make sure that tasks they were asked to perform for the research project were simple and easy to do and would not interfere with the tasks they were being asked to do as part of their routine commercial role during the voyage.

There was a clear recognition that research procedures would need to be streamlined and simplified. If AAVs or exporters felt that the research demands on AAV time during a voyage were excessive and interfered with the ability of an AAV to perform their routine commercial duties then this would potentially lead to lack of compliance with the research protocols and would interfere with the ability of the research project to achieve its goals.

4.2 Stage 1 – Development of procedures and protocols

The first stage of the project was largely focused on developing procedures and protocols for the implementation of research activities and engaging industry stakeholders through this process to ensure support for these protocols.

The primary output from all of the Stage 1 activities was the development of the Veterinary Export Handbook (W.LIV.0252) which was intended to serve as the single source of information and research protocols.

Activities conducted under other objectives in Stage 1 were all intended to provide relevant information into the design and development of the Veterinary Export Handbook (W.LIV.0252).

4.2.1 Veterinary Export Handbook (W.LIV.0252)

The final version of the Veterinary Export Handbook (W.LIV.0252) is reproduced in full in the companion volume of appendices that accompanies this final report.

The Veterinary Export Handbook (W.LIV.0252) was developed as a ring-bound A5 booklet, printed on water proof paper. It was intended to serve as a resource manual for AAVs working on commercial cattle export voyages enrolled in the project. It included standardised definitions and terms as well as standardised approaches for investigating sick and dead animals, including how to conduct a field necropsy. It contained a detailed protocol with examples of data collection forms for AAVs to follow for data collection as part of the project. It also included protocols for collection, labelling and storage on the ship of biological samples collected at necropsy for subsequent importation back into Australia. Completed forms from investigations of cattle deaths and samples and digital photographs of animals were imported back into Australia and subsequently transported to the pathology laboratory at the Department of Agriculture and Food Western Australia (DAFWA) for examination by veterinary pathologists.

The Veterinary Export Handbook (W.LIV.0252) had three primary objectives:

1. To provide a resource for AAVs in a simple, easy to carry form that covered common diseases likely to be seen in export cattle, and that provided brief information on each disease including diagnosis and treatment.
2. To provide standardised procedures and terminology for investigation of sick and dead cattle (including how to conduct a necropsy) so that records and reports from different veterinarians and different voyages would be more consistent and comparable.
3. To provide protocols and template forms for collection of information and data on sick and dead cattle and for collection of biological samples (swabs and tissues) from sick and dead cattle, as part of the research project (W.LIV.0252)

The Veterinary Export Handbook (W.LIV.0252) was reviewed and updated periodically through the course of the project, primarily in response to feedback from AAVs and exporters. The final version of the Veterinary Export Handbook (W.LIV.0252) was labelled as V16 and dated 31 October 2011.

The main changes in the Veterinary Export Handbook (W.LIV.0252) related to the research protocols for collection of samples and recording of information on sick and dead cattle during a voyage. Initial protocols called for more detailed collection of information and samples on sick and dead cattle and asked that necropsy be performed on as many of the dead cattle as possible. These protocols were deemed to be non-practical and were changed as a result of feedback arising from the pilot voyages to a more streamlined protocol that included a daily health report.

The Veterinary Export Handbook (W.LIV.0252) included information on research activities (sample and data collection for the W.LIV.0252 project) that was only relevant while the research project was active. At the time the Veterinary Export Handbook (W.LIV.0252) was produced it was anticipated that a more general handbook would be produced towards the end of the project as a generic resource for the livestock export industry.

A separate handbook was subsequently produced in 2012 as a generic resource for the livestock export industry. [10] This ring-bound printed book was titled ***Veterinary handbook for the live export industry*** and it provided a detailed reference of common conditions in export animals that included conditions of dairy and beef cattle as well as sheep and goats. The content of the ***Veterinary handbook for the live export industry*** was developed by starting with the material from the Veterinary Export Handbook (W.LIV.0252) and adding or modifying as needed to produce a generic resource covering cattle, sheep and goats.

In 2014 the content of the hardcover ***Veterinary handbook for the live export industry*** was released as a web resource, titled ***Veterinary handbook for cattle, sheep and goats*** [11] and as an app for download on mobile devices.[12],[13]

[10] Jubb and Perkins (2012); Perkins and Jubb (2012)
[11] http://www.veterinaryhandbook.com.au/
[12] http://minister.agriculture.gov.au/joyce/Pages/Media-Releases/new-vet-handbook-app-will-further-improve-animal-welfare.aspx
[13] http://www.livecorp.com.au/research-development/reports/veterinary-handbook-for-cattle-sheep-and-goats

The Veterinary Export Handbook (W.LIV.0252) is referred to with the project identification code (W.LIV.0252) purposefully included in the title to clearly differentiate this project-specific handbook from the broader more generic electronic resource.

A brief summary is provided here of the protocols incorporated into the Veterinary Export Handbook (W.LIV.0252) that related to collection of research information and biological samples for this project.

4.2.1.1 Daily health report

At the end of each voyage day, AAVs were asked to complete a ***Daily Health Report*** form that was intended to provide a daily record of standardised information about general health for cattle on the voyage. The ***Daily Health Report*** template was described and explained in the Veterinary Export Handbook (W.LIV.0252) and blank template forms were provided for every AAV to complete during each voyage.

Preliminary discussions with AAVs indicated that most AAVs collected observations in a variety of formats (mostly notes recorded in some form of pocket book) during the day and they would draw on this information in contributing to the formal daily voyage report.

At the time this project started (late 2009), AAVs were contributing material into the daily voyage report but did not generally retain copies of the daily voyage report. Our enquiries suggested that it would not be possible for the project team to easily obtain copies of all daily voyage reports from all enrolled voyages. This led to the need for the ***Daily Health Report*** form.

The ***Daily Health Report*** designed as part of this project was intended to provide a template for AAVs to collect information in a standardised way that allowed them to contribute as required to the daily voyage report while retaining the ***Daily Health Report*** form for their own record keeping purposes and for the project. At the end of the voyage, a copy of each day's ***Daily Health Report*** would then be included in material sent back to the research project team.

The ***Daily Health Report*** template is provided in the companion volume of appendices that accompanies this final report. It is presented as part of the Veterinary Export Handbook (W.LIV.0252).

4.2.1.2 Necropsy / dead cattle report

A copy of the ***Necropsy / dead cattle report*** is provided in the companion volume of appendices that accompanies this final report. It is presented as part of the Veterinary Export Handbook (W.LIV.0252).

AAVs were asked to complete a ***necropsy / dead cattle report*** form for every dead animal as a record of death.

A standardised set of baseline information was requested in this form for every dead animal including information about the animal (ID, breed, age, sex, estimated weight, condition score, signs displayed before death, treatments, location of the animal at time of death, predisposing factors or events and a tentative cause of death).

Where dead cattle were subjected to a necropsy, the ***necropsy / dead cattle report*** form included scope for necropsy information to be recorded as well. This included a diagram of right and left lung anatomy where the AAV could circle locations for any lung pathology at gross necropsy and space for recording text descriptions of gross necropsy findings. There was also a list of necropsy samples where AAVs could circle those samples that had been collected from each necropsy.

The Veterinary Export Handbook (W.LIV.0252) included standardised protocols for selection of animals to post-mortem, guidelines on the number of animals to post-mortem when multiple deaths occurred, an outline of how to perform a gross necropsy in the field, and procedures for collecting, labelling and storing biological samples from animals at necropsy.

Procedures recognised that not every dead animal could be necropsied because of time constraints and other factors.

Where multiple deaths occurred from the same disease syndrome and around the same time (within 1-2 days), AAVs were asked to necropsy three representative animals only.

If the same disease or syndrome continued to be associated with deaths over subsequent days or later in the voyage, AAVs were asked to conduct additional confirmatory necropsies at a lower rate (one per 1-2 days) from those animals.

If at any stage there was suspicion of a different disease syndrome, then AAVs were asked to conduct necropsies of three representative animals i.e. follow the same general protocol as above.

Necropsy protocols were described under two different approaches: targeted and comprehensive.

Targeted necropsy referred to a partial necropsy designed to provide gross examination (and description) of the minimum set of organs and tissues to characterise most causes of death. It involved examination of chest and abdominal cavities in all cases and additional tissues only if indicated by history, clinical signs or initial findings.

A comprehensive necropsy referred to a complete necropsy and a comprehensive sampling strategy. It was to be performed when there was no obvious cause of death to ensure maximal likelihood of subsequent laboratory examination identifying the cause of death.

Protocols also called for biological samples to be collected from animals at necropsy. Types of samples and storage methods were based on requirements necessary to meet government importation restrictions for biological material given that these samples were to be imported back into Australia for examination by veterinary pathologists as part of the research project to establish causes of death.

A set of core samples was defined that were to be collected from every necropsy and that would form a minimum set of samples necessary to differentiate the major expected causes of death.

Where certain defined diseases were suspected based on ante-mortem signs or gross necropsy changes, additional samples were defined for each disease to provide increased confidence in diagnosing or ruling out these specific conditions as causes of death.

Finally, a comprehensive sampling protocol involving a large list of tissues to be sampled was provided for those situations where a comprehensive necropsy was performed.

4.2.1.3 Additional procedures

A variety of additional information was provided in the Veterinary Export Handbook (W.LIV.0252) to provide resource material to guide AAVs in collecting and storing information and samples during a voyage.

Digital photographs were recognised as an excellent ancillary source of visual information about sick and dead animals. Information was provided about how to take, label and store digital images from sick animals and from necropsies.

A standardised veterinary kit was defined for the purposes of this project (list of equipment considered necessary to perform the tasks described in research protocols). The list was reconciled against equipment already considered to be standard and supplied by either the AAV or by the ship. All additional items defined in the list were then provided by the project and loaded onto each participating vessel at the time cattle were loaded. This ensured that AAVs had all the equipment required to complete any procedures they were being requested to do as part of the project. The equipment included sample collection and storage items and a range of more specialised necropsy equipment to ensure that all aspects of a necropsy could be undertaken to the same standard.

Advice was provided on how to label and store samples on board the vessel and how to package up all the samples and completed paperwork at the end of the voyage. Forms were provided for the AAV to complete at the end of the voyage in preparation for importation of the stored samples back into Australia. Stored samples and completed import forms were then held on the vessel until it docked in an Australian port to begin another voyage. At that time a member of the project team would visit the vessel and collect the stored samples and related paperwork and process them through quarantine for importation into Australia.

Forms were also included in the kit for AAVs to complete as an inventory assessment of the equipment kit. When the vessel docked back in an Australian port the visiting project team member could then review the equipment list and replenish or replace equipment as necessary to ensure that the vessel had a full set of equipment before the start of the next voyage.

4.2.2 Results from other objectives under Stage 1

Data requirements for collecting information on healthy, sick and dead cattle (Objective 2) were developed in conjunction with Objective 1, including forms designed to facilitate collection of information by on-board veterinarians. Outputs from these activities were incorporated into the Veterinary Export Handbook (W.LIV.0252).

Necessary contents of a veterinary kit (Objective 3) were determined and systems developed for management of the kit on board export vessels including how to replenish and maintain the kit. Systems were also developed for importing samples from sick or dead cattle back into Australia for examination by pathologists as part of the project. Details of the contents and tools contained in a veterinary kit are defined in a section of the Veterinary Export Handbook (W.LIV.0252).

A review was conducted of the responsibilities of on-board veterinarians (Objective 4) based on discussion with industry stakeholders, review of relevant regulations and copies of typical contracts or letters of agreement between employers (exporters) and veterinarians. The purpose of this process was to understand what activities AAVs were already being expected to undertake during routine export voyages. Then as research protocols were being developed for this project, any activities that AAVs might be asked to undertake for the research project could be mapped to existing tasks to ensure that the research project was not asking AAVs to undertake any new or different tasks.

When we started this engagement process we were unsure whether AAVs and stockpersons might be directly involved in collection of data and information for the research study or whether this involvement might be limited to AAVs. Initial discussion with industry indicated that all voyages being considered for enrolment in the study would have an accompanying AAV and that AAVs would be the preferred focal point for collaborative involvement in research activities. Our focus was then directed to understanding AAV responsibilities primarily to ensure that project-related tasks would be relevant to routine job requirements already being undertaken by AAVs.

The full report titled ***Review of job description for Australian Government Accredited Veterinarians (AAVs)*** is presented in the companion volume of appendices that accompanies this final report.

Job descriptions and contractual agreements between AAVs and stockpersons and an exporter represented commercially sensitive agreements. We were not able to obtain job descriptions.

A key finding of this component report was that almost all research tasks that AAVs would be asked to undertake for this project were tasks that were already clearly defined as AAV responsibilities on routine commercial voyages unrelated to W.LIV.0252. The only changes were related to minor refinements in how observations were made, how data and information might be collected for research purposes and protocols for collection, labelling and storage of biological samples from animals at necropsy.

A training needs assessment was conducted for accredited veterinarians and stockpersons participating in disease investigations on cattle export vessels (Objective 5). In preparing procedures and protocols it was recognised that some level of training would be required to ensure that AAVs (and stockpersons) were familiar with the procedures and protocols. The training needs assessment considered the following issues:

- What skills and knowledge are required to deliver the tasks to the required standard as defined in the Veterinary Export Handbook (W.LIV.0252);
- What were the existing skills and knowledge among AAVs and stockpersons;
- What training courses and materials were already available;
- What constraints were there to running training; and
- What were the training options.

The full report titled ***Training needs assessment*** is presented in the companion volume of appendices that accompanies this final report.

The training needs assessment report identified a range of options for development and delivery of training to AAVs and stockpersons both for the purposes of the research project and more broadly for improved animal health management over the longer term.

In preparation for Stage 2 (Pilot voyages), a one day training workshop was delivered by project team members at the University of Sydney. This training program was attended by two AAVs and involved hands-on training in how to conduct a field necropsy and collect samples and a classroom review of the procedures and protocols outlined in the Export Veterinary Handbook (W.LIV.0252). This ensured that AAVs participating in the pilot voyages had received training in the research procedures and in the standardised approaches to investigating and describing sick and dead animals.

Subsequently a separate project was initiated to formally develop and deliver a training package and a professionally filmed DVD on field necropsy procedures. [14]

The project team reviewed existing approaches to collection of data and information and how data/information was managed on board ship (Objective 6). Existing systems were predominantly paper-based though changes associated with NLIS appear to have resulted in an increase in potential for electronic data systems.

The project team concluded that a paper-based recording system was most effective for the pilot voyages.

The project team was aware of interest about the development of a computer/web-based or hand-held system for implementation across the export industry that is capable of managing data and information for cattle, sheep and other animals that are exported. It was recognised that development of a broad, multi-species system was not intended for completion as part of this project but that there would be an opportunity to discuss issues related to management of data. The project team agreed to develop a prototype hand-held device for use by on-board veterinarians as part of the current project. This system will be developed largely as a prototype and to inform specification of a broader information management system (IMS) that may be developed to cover all species of animals that are exported. This is discussed later in the report (see Chapter 10).

The standardised approach developed for collection of research data was incorporated directly into the Veterinary Export Handbook (W.LIV.0252).

Discussions were held amongst the project team on economic frameworks (Objective 7) for assessment of impact of morbidity and mortality and benefits of reducing adverse health events. The conclusion was that economic analyses could be applied in the final report provided that data was collected on measures such as counts of mortalities and morbidities, and information on treatments and their impacts on health outcomes.

[14] Perkins, Jubb, and O'Hara (2012)
[14] Moore (2014)

5 Results – development of qPCR assays

Readers are referred to Dr Jo Moore's PhD thesis for more detail on this section. [15] This section of the report will provide a brief overview of the processes.

The objective of this part of the work was to develop and validate quantitative polymerase chain reaction and reverse transcription polymerase chain reaction (qPCR) assays for the detection of viruses and bacteria known to be important in the pathogenesis of bovine respiratory disease (Bovine coronavirus (Betacoronavirus 1), Bovine herpesvirus 1, Bovine parainfluenza virus 3, Bovine respiratory syncytial virus, Bovine viral diarrhoea virus, *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma bovis*, *Histophilus somni*) in swab and tissue samples. Where possible, assays were duplexed to reduce the number of tests required.

Due to quarantine restrictions imposed by the Australian Government, biological material collected during voyages could not be used for microbial culture or imported back into Australia as live cultures. In addition, samples needed to be robust in the face of variable storage conditions on-board vessels and a significant time period (1-6 months) between sample collection and processing. Given these constraints a decision was made to develop qPCR assays using samples that were stored in a way that ensured inactivation of all biological material before samples were imported back into Australia.

Viral samples used for test validation were obtained from clinical or research samples retrieved from DAFWA storage for all viruses except BRSV. Human respiratory syncytial virus was kindly provided by Dr Tim Mahoney (Department of Employment, Economic Development and Innovation, Queensland) as a positive control for the BRSV qPCR.

Bacterial DNA samples from *M. haemolytica, P. multocida, M. bovis* and *H. somni* were kindly provided by Sam Hair from isolates held in the DAFWA bacteriology laboratory.

Validated qPCR tests were developed for all of the pathogens of interest for this project.

Tests for BoHV-1, BPIV-3, *M. bovis*, *M. haemolytica*, *P. multocida* and *H. somni* showed a detection limit (analytical sensitivity) of less than 10 viral/bacterial gene copies, while tests for BCoV, BRSV and BVDV showed detection limits of 20, and 100-200 copies respectively. The lower analytical sensitivity of BCoV, BRSV and BVDV assays may be due to interactions between primers pairs in the duplex. However, the analytical sensitivity of these two assays was still higher than virus isolation or antigen capture ELISA for detection of viral genomes.

The qPCR tests have provided a sensitive, rapid tool for detection of nucleic acids from specific viruses and bacteria of interest in nasal and tissue swab samples. These tests have been extensively applied to samples collected through the course of the field work phases of this project.

6 Results - Stage 2 – Pilot voyages

Two voyages were enrolled under Stage 2, departing Fremantle in March 2010 and May 2010.

There were 16 deaths on the first voyage and all 16 animals were subjected to a post mortem examination as per project protocols. Samples and associated paper work were stored in the drug room on board the vessel and in an esky in a refrigerator in the drug room.

The second voyage departed Fremantle on 10 May 2010. There were a total of 13 deaths during the voyage. Nine of these deaths occurred at sea and eight of these animals were subjected to a necropsy as per project protocols. One animal was not necropsied because of advanced decomposition and an additional four deaths occurred while the vessel was in port (animals euthanased because they were unable to be unloaded) and it was not possible to do necropsies while the vessel was at port.

Detailed findings on causes of death and risk factors for mortality arising from data collected during the pilot voyages are not presented in this section of the report. The data from these two voyages has been added to the other voyages completed as part of Stage 3 and all results are described together in later sections of this report.

This section is focused on feedback obtained from the pilot voyages that related to the procedures and protocols for the project. Feedback was sought directly from AAVs, exporters, MLA/LiveCorp and members of the project team. A stakeholder meeting was held in July 2010 and additional feedback was obtained at this meeting.

The training day and the necropsy kit developed for the project were both well received. In particular the bucket system used to store and carry the equipment (developed by Dr Tristan Jubb) was noted as an innovative approach to ensuring all the equipment required to perform a complete necropsy could be carried around a vessel by a single person.

Mr Greg Norman (DAFWA member of the project team) noted that there were no problems experienced in either getting the kit on board the vessel prior to departure, or in retrieving and importing samples and paper work at the end of the return voyage. The samples were well packaged and appropriate paper work had been completed by the AAV prior to departing the boat at the destination port. Areas for improvement included in particular the need for the AAV to complete an inventory of the kit and consumables at the end of the voyage to get information back to Greg about what equipment is required to replenish the kit. An inventory form was added to the kit to allow the AAV to rapidly work through a tick the box review process at the end of the voyage and indicate which equipment and stores needed replenishing or replacing.

Pathology team members reported that samples arrived in excellent shape, necropsy/dead cattle forms were completed for each necropsy and that digital photographs of necropsies were particularly useful when assessing and relating gross and histologic changes. There was very good agreement between the initial diagnosis filled in by the AAV at the time of the gross necropsy and the final diagnosis based on laboratory assessment of samples.

AAVs provided electronic files of the necropsy/dead cattle report indicating that the project needs to provide the capacity for AAV to complete forms electronically as well as in paper form. This was implemented in subsequent voyages.

Concerns were raised about a number of issues relating to project activities and in particular about how to improve the project going forward. The main areas of concern included:

- The amount of time spent by the AAV in doing necropsies – both time spent doing each necropsy and the total time given that there is an expectation that all dead cattle would be necropsied (or as many as possible).
- The amount of time spent by the AAV in completing project paperwork and particularly the Daily health report.
- Concerns that the project was focussing too much on voyages and necropsies and that there should be more focus on the things that may be happening to animals in the assembly feedlot and even on the property of origin that may influence likelihood of disease occurring during the voyage.
- Concerns that for a range of reasons (including treatment/vaccination of animals prior to loading and selection of animals etc) voyages may not have many deaths and the project may therefore not generate much value for the industry.

The following changes were implemented as a result of feedback following completion of the two pilot voyages.

1. Changes to necropsy procedures described in the Veterinary Export Handbook (W.LIV.0252) and implemented for all subsequent voyages.

   Necropsy procedures were simplified to make it easier for the AAV to perform necropsies and collect samples. In most situations the AAV was expected to complete a targeted necropsy only and collect a relatively small set of biological samples (core samples) and additional samples defined for selected conditions.

   Necropsy procedures were also simplified to allow for fewer necropsies to be required. Where there were multiple deaths from the same syndrome, a sample of 3 representative animals should be necropsied to establish the cause of death and other animals dying with the same syndrome could be assigned to that cause of death without a necropsy. If deaths from the same syndrome continue over several days then occasional additional necropsies (one every 1-2 days) would be recommended to continue to collect good information. These changes were implemented to reduce the time requirement for busy AAVs.

2. Changes to the data collection forms in the Veterinary Export Handbook (W.LIV.0252).

   There were concerns over the amount of time taken to fill in forms and particularly the *Daily Health Report Form*. All forms were reviewed and major changes were made to the *Daily Health Report Form*. The form was altered to mainly ask for simply summary information in abbreviated form and by asking the AAV to circle or tick options. Every effort was made to eliminate questions that might involve information that does not change over time or where the information can be obtained from other sources. The intent was to simplify the form so it could be completed each day with minimal time and effort.

Minor changes were also made to the *Necropsy / dead cattle report,* again to provide more tick and circle options and make the forms simpler and easier to fill in.

Electronic versions of the forms were provided in Microsoft Excel® or Word® to make it easier for AAVs to fill in forms using a laptop and printed copies were provided for AAVs that preferred to fill in paper forms.

3. Clarification of approach to collecting additional voyage data (denominator data on animals loaded, daily climate and location data etc).

   A draft list of additional data of interest to the project was produced and circulated to exporters (Figure 1). Listed variables were included because they were deemed to be important for description of morbidity and mortality during the export process and also for investigation and assessment of potential risk factors.

Animals arriving at feedlot	**Assembly feedlot period**	**Voyage**
Consignment or mob identifier	Morbidities	Voyage ID
Count of animals	Date	Port of loading
NLIS tag numbers	Disease	Vessel name
PIC or property of origin	Treatment	Exporter
Breed	Recovery(?)	Departure date
Age	Samples(?)	Destination ports
Sex	Mortalities	Animals loaded
Dated loaded on property	Date	Consignment ID
Date of arrival at feedlot	Cause	Count
Feedlot identification	Necropsy(?)	Sex
Voyage ID	Samples(?)	Age
Arrival weight	Vaccination	Breed
Scale location	Treatments	Weight/Class
	Feed information	counts by deck/hold/pen
	Feedlot identification	Daily records
	Voyage ID	temp Wet/Dry (each deck)
	Date of loadout	humidity (each deck)
	Loadout weight(?)	deck conditions
	Consignment or mob identifier	feed/water consumption
	Count of animals loaded	sea/climate
	Discards (animals not loaded)	hospital pen report
		Unload data at each destination
		Destination port
		Animal consignment ID
		Count unloaded
		Unload date
		Treatments/drugs used during voyage

Figure 1: Summary of data requirements for additional data to be sourced from exporters

4. A decision was made to explore options for accessing additional datasets relevant to the project.

   i. There was interest in describing animal movement patterns within Australia for animals that were destined for live export from Western Australian ports. A process was initiated to access de-identified and aggregated data from the cattle NLIS database in Western Australia to describe general patterns of animal movements for animals destined for export. This approach resulted in adding spatial analyses to the results.

   ii. There was interest in applying advanced analytical methods to summary data on livestock exports used to produce annual summary reports for the livestock export

industry. A process was initiated to obtain permission from industry bodies to access stored datasets managed by DAFWA for the purpose of generating annual industry summary reports.

5. Extension of project activities into assembly feedlot and property of origin. There was general recognition that events occurring prior to the voyage could influence the risk of morbidity or mortality during the voyage. There was general support for extension of the project to the assembly feedlot and depending on feasibility and cost, to property of origin. This issue resulted in a contract extension to the project and the addition of objectives 11 to 13 under Stage 4 (See Chapter 2).

7 Results - Stage 3 - Collection of data on 24 months of voyages

7.1 Data sources

Data was sourced from several different sources during the course of this project including from exporters, AAVs, Department of Agriculture website (parliamentary summary reports of livestock export voyages) and from the Shipboard Mortality Database (SMDB). There were occasions where the summary counts of livestock loaded or unloaded for a particular voyage and the count of livestock mortalities were not numerically identical in all of these different sources. Discrepancies were assumed to be the result of errors in counting or recording. Where counts were not the same in data from different sources, an attempt was made to check varying sources and where possible adjust estimates based on the most plausible and reliable source in order to maintain a baseline dataset that included consistent counts for cattle loaded onto each voyage and for cattle deaths during each voyage.

There was a single voyage with a discrepancy of 1 animal in counts of mortalities between datasets from different sources. In this case, the most reliable source was considered to be a mortality investigation report accessed from the Department of Agriculture website and this count was then applied to all other datasets for this voyage.

There were two voyages with discrepancies in total cattle loaded. For one voyage the SMDB recorded a figure for total cattle loaded that was 1 animal higher than the figure recorded in all other sources (discrepancy of 1 out of a total loaded of 11,537 cattle). For the other voyage the SMDB record was 35 animals higher (17,484) than records from other sources (17,449). In both cases the datasets used for all analyses were adjusted to reflect the lower total counts.

This approach was necessary because data was combined from different data sources into a single dataset for analyses on mortality during export. Adjustments were so small relative to total counts that the changes were considered unlikely to have any impact at all on interpretation of analytical results and the reason the changes were necessary was only for consistency of datasets and reporting.

7.2 Participating voyages

A voyage was defined eligible for participation in the project, if it met the following criteria: loading more than 1,000 cattle in Australia for a long haul voyage (≥ 10 days) to destinations

including the Middle East (Israel, Saudi Arabia, Bahrain), North Africa (Egypt, Libya), Turkey and the Russian Federation (Russia).

The first Stage 3 voyage was enrolled in August 2010 and voyages were enrolled through to September 2012. There was a short delay between the end of Stage 2 (voyage completed in June 2010) and the start of the first voyage under Stage 3. The delay involved time required to analyse and report on samples from the two pilot voyages and then to modify procedures and protocols in response to stakeholder feedback from Stage 2.

Data from the two pilot voyages was included with all Stage 3 voyages for analyses and reporting. The combined dataset therefore started with the first voyage under Stage 2 which sailed from Australia in March 2010 and concluded with the final enrolled voyage that sailed from Australia in June 2012.

Data on counts of livestock exported by voyage and numbers of deaths were obtained from the 6-monthly reports on livestock mortalities that are tabled in each House of Parliament by the Minister for Agriculture and are available for access on the Department of Agriculture website. These reports provide details of port of loading, date of loading, duration of voyage, total animals loaded and numbers of deaths. This data was used to identify those voyages occurring in 2010, 2011 and 2012 that met eligibility criteria. A total of 72 voyages were identified from this dataset, comprising 551,742 cattle exported from Australia to various destinations. Of these a total of 61 voyages occurred during the period when this project was active (March 2010 to September 2012).

Twenty voyages were enrolled in Stage 2 or Stage 3 and contributed information and samples relating to causes of mortality during export voyages. These 20 voyages included one voyage to China (voyage 19) that was enrolled as an opportunistic long-haul voyage even though it did not strictly meet all the eligibility criteria.

The 19 voyages participating in Stage 2 or 3 and meeting eligibility criteria represented 31% of the 61 eligible voyages during the period when the project was active. The total cattle loaded on the 19 voyages meeting eligibility criteria (188,336) represented 40% of the total cattle loaded on all 61 eligible voyages (474,360).

The period when this project was operating covered the period when live exports to Indonesia were stopped (mid 2011) and the implementation of ESCAS, initially for Indonesian exports and then for all livestock exports from Australia. There was considerable uncertainty associated with livestock exports during the latter part of the active period for this project and these issues are considered likely to have impacted on participation in the project.

Table 1: Summary information for voyages enrolled in the project from March 2010 to September 2012

Voyage code	Ship code	Year	Season	Load port(s)	Discharge country	Voyage duration (days)	Cattle loaded (N)	Mortality (n)	Mortality (% of N)
1	A	2010	Autumn	Fremantle	Israel	21.6	9,430	16	0.17
2	A	2010	Autumn	Fremantle	Libya	22.9	9,213	13	0.14
3	B	2010	Winter	Broome	Egypt	18.1	5,090	4	0.08
4	C	2010	Spring	Fremantle	Saudi Arabia	18.6	10,428	11	0.11
5	C	2010	Summer	Fremantle	Turkey	23.6	19,990	148	0.74
6	D	2010	Summer	Portland	Russia	31.3	3,994	21	0.53
7	E	2010	Summer	Fremantle	Turkey	34.9	16,255	25	0.15
8	C	2011	Autumn	Portland, Fremantle	Saudi Arabia, Turkey	34.9	17,449	52	0.3
9	F	2011	Autumn	Fremantle	Turkey	41.5	10,237	60	0.59
10	G	2011	Autumn	Portland, Fremantle	Russia	35.1	12,763	54	0.42
11	E	2011	Winter	Portland, Fremantle	Turkey	36.7	9,000	107	1.19
12	C	2011	Winter	Broome, Fremantle	Egypt	23.2	9,239	27	0.29
13	B	2011	Winter	Broome, Fremantle	Bahrain	19.9	1,350	6	0.44
14	A	2011	Spring	Adelaide, Fremantle	Turkey	34.1	4,274	15	0.35
15	E	2011	Spring	Adelaide, Fremantle	Turkey	31	12,256	61	0.5
16	F	2011	Summer	Fremantle	Israel	21.5	9,811	17	0.17
17	A	2012	Autumn	Adelaide, Fremantle	Turkey	33.6	7,811	39	0.5
19	B	2012	Autumn	Portland	China	18.3	5,880	8	0.14
20	E	2012	Autumn	Adelaide, Fremantle	Turkey	36.1	11,537	37	0.32
21	A	2012	Winter	Adelaide, Fremantle	Israel	29.5	8,209	21	0.26
							194,216	742	0.38

Table 2: Summary information for all voyages to Middle East and Russia departing in 2010, 2011 and 2012 with >1000 cattle loaded and the voyages that participated in this project

	All voyages to Middle East & Russia with >1000 cattle						Participating voyages					
Year	Season	Voyages (voy.n)	Duration (d)	Cattle loaded (N)	Mortalities (n)	Mortalities (% of N)	Mortalities Voy range for %	Voyages (voy.n)	Duration (d)	Cattle loaded (N)	Mortalities (n)	Mortalities (% of N)	Mortalities Voy range for %
2010	Summer	4	23 to 32	36,988	430	1.16	0.18 to 1.79						
2010	Autumn	4	22 to 40	25,247	37	0.15	0.1 to 0.17	2	22 to 30	18,643	29	0.16	0.14 to 0.17
2010	Winter	7	18 to 27	56,728	135	0.24	0.07 to 0.57	1	18 to 18	5,090	4	0.08	
2010	Spring	6	19 to 35	59,647	151	0.25	0.07 to 0.64	1	19 to 19	10,428	11	0.11	
2010-2011	Summer	9	17 to 32	73,096	287	0.39	0.05 to 0.74	3	22 to 31	40,239	194	0.48	0.15 to 0.74
2011	Autumn	4	30 to 41	49,733	177	0.36	0.12 to 0.59	3	34 to 41	40,449	166	0.41	0.3 to 0.59
2011	Winter	6	20 to 38	35,868	173	0.48	0.11 to 1.18	4	20 to 38	23,863	155	0.65	0.29 to 1.19
2011	Spring	8	19 to 36	45,611	129	0.28	0 to 0.5	1	27 to 27	12,256	61	0.50	
2011-2012	Summer	5	22 to 33	31,382	53	0.17	0.07 to 0.23	1	22 to 22	9,811	17	0.17	
2012	Autumn	5	20 to 36	30,216	110	0.36	0.06 to 0.5	3#	20 to 33	25,228	84	0.33	0.14 to 0.5
2012	Winter	7	18 to 29	60,952	96	0.16	0.05 to 0.26	1	28 to 28	8,209	21	0.26	
2012	Spring	7	17 to 35	40,394	55	0.14	0 to 0.17						
Total		72		551,742	1,841	0.33	0 to 1.79	20		194,216	742	0.38	0.08 to 1.19

includes one voyage to China

Records from the 6-monthly parliamentary reports were sourced and data summarised for those eligible voyages departing Australia during 2010-2012, with more than 1,000 cattle loaded and travelling to ports in the Middle East (including Turkey) and Russia. The right side of Table 2 shows summary information from the subset of eligible voyages that participated in the project.

For the 20 participating voyages, cattle were loaded out of four different ports across three states: Fremantle and Broome (Western Australia), Portland (Victoria) and Adelaide (South Australia). The majority of cattle were loaded from Fremantle. Cattle from southern ports (Portland, Adelaide, Fremantle) tend to be primarily of the *Bos taurus* subspecies while those from the northern port (Broome) are primarily *Bos indicus.* [16]

Cattle were loaded out of a single port for 9 voyages while split loadings were used on the remaining 11 voyages. For split loadings, cattle were loaded at Broome, Portland, or Adelaide and then the ship sailed 4–5 days to Fremantle where additional cattle were loaded. All voyages out of Adelaide were split loaded while the remaining ports had a mixture of single and split loadings.

The number of cattle loaded for any one voyage varied from 1,350 to 19,990 (Table 1). Thirteen of the 20 participating voyages had both sheep and cattle loaded on the vessel and the remaining seven voyages had only cattle. The number of sheep loaded on a vessel ranged from 14,000 to 92,000.

A total of seven different ships were used to carry livestock for the participating voyages with each ship making between 1-5 separate voyages during the study period.

Voyage duration ranged from 18 to 42 days (including sailing and discharge days) and the average voyage duration was 28.3 ± 7.4 days (mean ± standard deviation).

Of the participating voyages, 19 of the 20 discharged cattle at ports in two broad geographical zones: Middle East and North Africa (including Israel, Saudi Arabia, Bahrain, Libya, Egypt) and Turkey and the western Russian Federation. Turkey was the most common destination port, accounting for 45% of voyages and 47% of cattle on participating voyages.

The participating voyages are considered to be representative of the broader eligible group of voyages described in 3.7 Retrospective Data. Eligible voyages occurred across all seasons, voyage duration ranged from 17 to 41 days and mortality as a percentage of cattle loaded ranged from 0% (two voyages, each with between 1,500 and 2,000 cattle total) to a maximum of 1.79%. The general characteristics of participating voyages were similar to those of the broader eligible group.

7.3 Voyage mortality

Voyage mortality is commonly reported in industry statistics as a percentage of total cattle loaded. This is defined as ***voyage mortality percentage*** and is commonly reported for the overall voyage (based on all deaths reported during the voyage and with a denominator based on all cattle loaded onto the ship). Where detailed breakdowns are available it may be possible to report voyage mortality percentages for various subsets of cattle on a single voyage, based on port of loading for example or consignments, by livestock class or by property of origin group using NLIS tag numbers to identify property of origin based on PIC data.

[16] DAFWA (2009)

A consignment is generally interpreted as all animals loaded at one port and under the control of one exporter. A single voyage may contain multiple consignments loaded at multiple ports, and there may be more than one consignment loaded at a single port.

The 20 participating voyages were associated with 742 cattle mortalities from a total of 194,216 cattle loaded, producing an overall average voyage mortality percentage of 0.38% (95% CI from 0.36 to 0.41%), while the voyage-specific mortality percentage figures ranged from a low of 0.08% to a high of 1.19%.

One of the problems with voyage mortality percentage, as a performance measure, is that it does not account for variation in voyage duration. If two voyages loaded the same number of cattle and had the same risk of mortality for each day of the voyages but one voyage was several days longer in duration than the other, then the longer voyage would have a higher mortality percentage than the shorter voyage because it was longer in duration. It is also possible that a shorter voyage could have a considerably higher real mortality risk per voyage day, but end up with a lower voyage mortality percentage than a longer voyage because it was shorter in duration.

A different estimation method for calculating mortality can be used to adjust for voyage duration. This involves estimation of ***voyage mortality rate***. Voyage mortality rate uses the count of deaths as a numerator (as does the voyage mortality percentage) but it uses a different denominator. The denominator is a measure of animal-time at risk. Animal-time at risk is based on the average number of animals at risk of dying multiplied by the duration in days for the voyage. The average number of animals at risk of dying during a voyage is estimated by the number of animals loaded at the start of the voyage minus half the number of animals that died during the voyage. This adjustment is to account for the fact that once an animal dies, it needs to be removed from the denominator of animals at risk. Mortality rates estimated in this way are adjusted for time at risk and this allows direct comparison of mortality rates from different voyages and also comparisons between export mortality rates and those derived from other sources such as on-farm or feedlot mortality rates.

An added complication in estimating voyage mortality rate resulted from voyages where animals were loaded at more than one port in Australia (split voyages). More than half the voyages were split voyages. In a split voyage the overall voyage duration may be recorded as the number of days from the first load port to the destination. This does not account for the fact that the actual voyage length on the same ship will be different for animals loaded at different ports. Animals loaded at Adelaide will have a voyage duration several days longer than animals loaded onto the same ship at Fremantle and travelling to the same destination port. Detailed data from the SMDB was used to note the number of animals loaded at each port for participating voyages and the voyage length from port of loading to destination for each load port. Using SMDB data for voyage length and animals loaded by load port allowed accurate estimation of animal-days-at-risk for use in voyage mortality rate calculations.

The average ***voyage mortality rate*** derived from Poisson regression modelling involved the same count of total deaths (n=742). The average animal-time at risk over all 20 voyages was 5,358,883 cattle-days. The average voyage mortality rate was 0.138 deaths per 1,000 cattle days (95% confidence interval from 0.128–0.148). Figure 2 shows the range of voyage-specific estimates for each of voyage mortality percentage and voyage mortality rates.

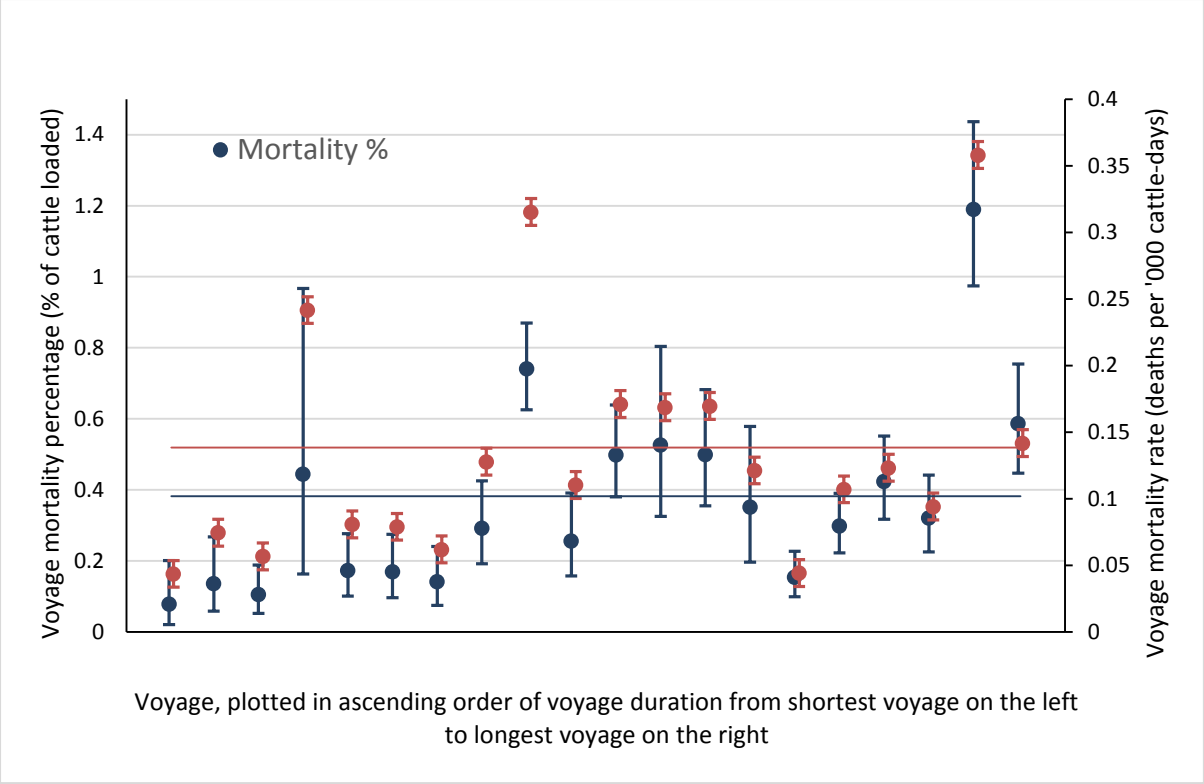

Figure 2: Plot of voyage mortality percentage (blue dots) for each voyage, arranged in order on the horizontal axis from shortest voyage duration to longest, and mortality rate (orange dots) for the same voyages. Bars represent the 95% confidence interval for each estimate. Horizontal lines represent the mean for mortality % (blue line) and mortality rate (orange line).

The reason for showing the two different mortality estimates side by side is to illustrate the fact that they are measuring slightly different attributes of mortality risk. It is not appropriate to compare mortality percentage estimates directly to mortality rate estimates. The importance of the two different measures is how they allow relative assessment of mortality risk in different voyages, i.e. when comparing mortality in one voyage to mortality in another voyage.

Mortality percentage is a crude measure that does not take any account of variation in voyage duration. The shortest voyage was 18 days in duration and the longest voyage was 42 days, more than twice as long as the shortest voyage. Where voyages are of the same (or very similar duration) then mortality percentage is a simple and effective way of comparing the mortality in one voyage to that in another. Where voyages are different in duration, voyage mortality percentage estimates are difficult to directly compare because two voyages may have the same underlying risk of mortality occurring on each day and have different mortality percentages simply because they had different durations. In this situation a longer voyage would be expected to have a higher mortality percentage because the mortality risk has been applied to the animals on the ship over a longer period of time.

Figure 2 also shows some association between voyage duration and mortality percentage with the shortest voyages also showing the lowest mortality percentage estimates.

Mortality rate estimates show a similar general pattern to the mortality percentage estimates, however there are some exceptions where individual voyages show a mortality rate that is relatively higher compared to other voyages than the mortality percentage.

Mortality rate estimates are able to be directly compared from one voyage to another even when the voyages have different durations, because duration is explicitly incorporated into the estimate of mortality rate (deaths per 1,000 cattle-days of voyage). A ship carrying 5,000 cattle over a voyage of 20 days will accumulate approximately 100,000 cattle-days at risk. A second ship carrying 5,000 cattle for 40 days of voyage will accumulate 200,000 cattle days at risk. When mortality rate is expressed as deaths per 1,000 cattle-days at risk, the specific mortality rate estimates from the two voyages have the same units and can be directly compared.

Poisson regression modelling of voyage mortality rate was used to screen other factors for association with voyage mortality rate. Models were run with ship added as a fixed effect to adjust for clustering at the ship level.

There was significant variation in voyage mortality rate between voyages ($p < 0.001$) which was expected and may be interpreted as indicating that there are likely to be other explanatory factors that may be influencing why some voyages have higher or lower mortality rates. Additional analyses in this and subsequent chapters are used to try and tease out possible factors that may explain some of the variation in voyage mortality rates and to use these findings to explore possible risk mitigation measures.

Date of departure from Australian ports was used to assigned voyages to a season and then season of departure was explored as a possible explanatory factor for voyage mortality rate (Table 3).

Table 3: Mortality rate estimates by season of departure from Australia derived from a Poisson regression model with season and ship added as fixed effects and adjusted for clustering at the voyage level, se=standard error, CI=confidence interval.

		Mortality rate		95% CI	
Season of departure	Number of voyages	deaths per '000 cattle-days	se	Lower	Upper
Autumn	8	0.107	0.014	0.080	0.134
Spring	3	0.120	0.038	0.044	0.195
Summer	4	0.133	0.061	0.014	0.251
Winter	5	0.201	0.066	0.071	0.331

Voyage mortality rate was higher in Winter and Summer than in Autumn and Spring.

When statistical tests were used to compare each season mortality rate to each other season, the results depended on whether the statistical model was adjusted for clustering or not. In a screening model unadjusted for possible effects of clustering, there were significant differences in mortality rate between seasons with mortality in Summer and Winter being significantly higher than in Autumn and Spring ($p<0.05$). When models were adjusted for clustering at both ship and voyage levels, all comparisons became non-significant ($p>0.05$) though there was a tendency for mortality rate in Winter to be statistically higher than in Autumn ($p=0.084$).

These findings reinforce the fact that clustering is likely to be an issue. Clustering is introduced in the Methodology section and is discussed in more detail in the discussion section. In addition, there are likely to be constraints on results imposed by the relatively small sample size (20 participating voyages). The combination of these effects is that some differences may be classified as statistically non-significant even though they may have genuine biological importance. The fact that mortality rate in Winter was almost double that reported for Autumn does suggest that Winter departing voyages may be dealing with an elevated mortality risk compared to voyages departing in other seasons.

Voyages were classified by load port and whether or not the voyage was a split port loading or not. Data on numbers loaded, mortalities and time at risk were estimated separately for each load-port component within each split-port voyage. A split port voyage therefore had at least two rows of data – one for cattle loaded at each port – while a single port voyage had only one row of data. Figure 3 shows mortality rate estimated by load port and load type (split load vs single port of loading).

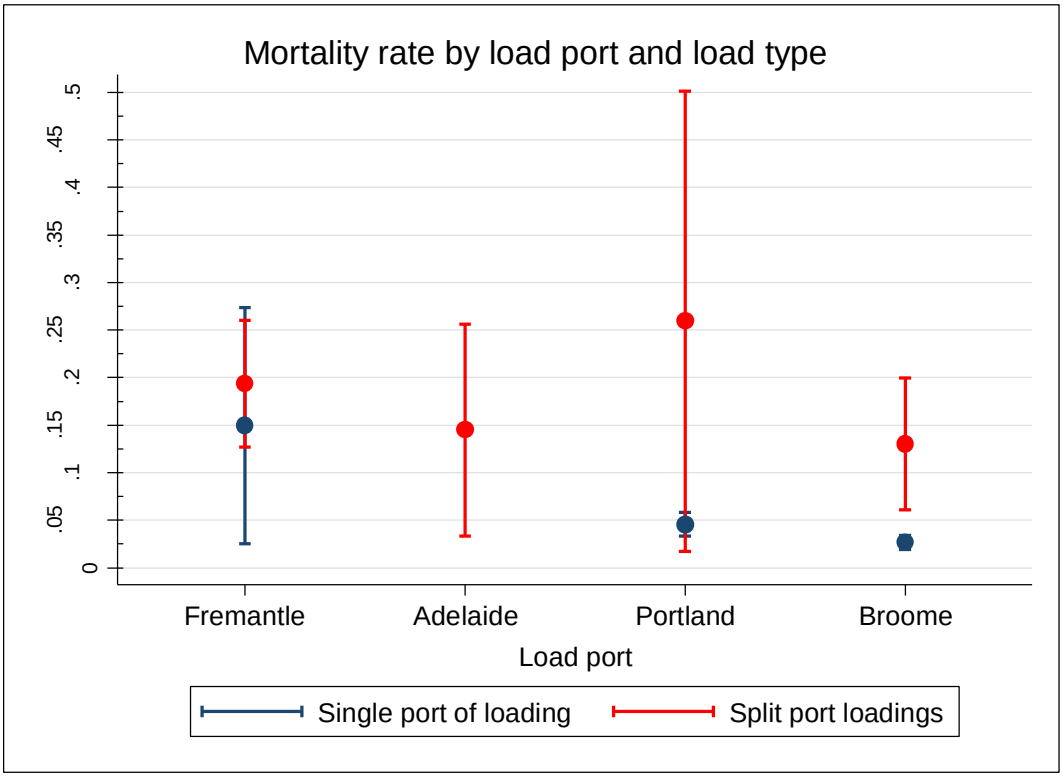

Figure 3: Plot of mean voyage-level mortality rate estimates arranged by load port and whether or not the voyage involved split-port loading. Blue circles represent the mean mortality rate for voyages with a single port of loading and orange circles represent voyages with a split port of loading. Bars represent 95% confidence intervals.

Table 4: Mean voyage mortality rate estimates arranged by load port and whether or not the voyage involved split-port loading. Within each load port a pairwise test was done to compare mortality rate for split-load voyages vs single port voyages based on mortality rate for cattle loaded from that port. se = standard error, CI = Confidence Interval

			Mortality rate		95% CI		
Port	Split load	Number of voyages	deaths per '000 cattle-days	se	Lower	Upper	p-value
Fremantle	No	7	0.150	0.064	0.025	0.274	0.600
Fremantle	Yes	10	0.194	0.034	0.127	0.260	
Adelaide	No	0					NA
Adelaide	Yes	5	0.145	0.057	0.033	0.256	
Portland	No	2	0.046	0.007	0.033	0.059	<0.001
Portland	Yes	3	0.259	0.123	0.017	0.501	
Broome	No	1	0.027	0.004	0.019	0.034	<0.001
Broome	Yes	2	0.131	0.035	0.061	0.200	

Three of the four ports had loaded cattle onto split-port loadings and single port loadings. All split port loadings involved cattle being loaded first at either Adelaide, Portland or Broome and then the ship sailed to Fremantle where additional cattle were loaded. Adelaide had no single port loadings and all voyages that involved cattle loaded in Adelaide were split-port voyages with additional cattle loaded at Fremantle. Once the second loading was completed in split-port voyages, the ship then sailed for its first overseas destination port. Single port loadings involved a voyage where a ship loaded all cattle for that voyage at one port and left directly for the first overseas destination port.

At all three ports where comparisons were possible, mortality rates for cattle loaded at that port and sailing on voyages with split-port loading were higher than the mortality rate in voyages from the same port and with single port loading. The difference was not significant for voyages from Fremantle ($p=0.6$) and was significant for voyages from Portland ($p<0.001$) and Broome ($p<0.001$).

Within those voyages with split-port loading, there was no statistical difference in mortality rates for cattle loaded from any of the four ports. This appears to be due to relatively large standard errors and wide confidence intervals as reflected in Figure 3.

Within those voyages involving only single port loading, mortality rates for cattle loaded from Broome and Portland were significantly lower than mortality rates for cattle loaded from Fremantle ($p<0.05$).

The two voyages from Portland with single-port loading, both involved dairy heifers and/or dairy cows, presumably being exported as breeding animals. Exports of higher value, breeding dairy cattle, tend to be managed differently to exports of beef cattle that are predominantly being exported as feeder or slaughter animals. Because the animals and their management during the voyage are different, it is not possible to directly compare voyage mortality rates between beef and dairy export voyages.

There was only one voyage involving a single port of loading and that sailed from Broome. This voyage involved mainly beef steers travelling in Winter to Egypt. It is not possible to be confident of comparisons where one estimate was generated from a single voyage, because

that voyage may not necessarily be representative of the group under consideration. This voyage - from Broome to Egypt -- involved one of the shortest voyage durations (18 days) and may have involved mainly *Bos indicus* genotype whereas animals loaded in southern ports (Fremantle, Portland and Adelaide) may have been more likely to involve *Bos taurus* genotype cattle. Records of breed were not available for cattle loaded on participating voyages.

The same Poisson model was then run without any adjustment for split-loading to allow comparison of load port. The results (Figure 4) suggest that mortality rates are highest in those cattle loaded at Portland, however none of the comparisons were statistically significant (p>0.05).

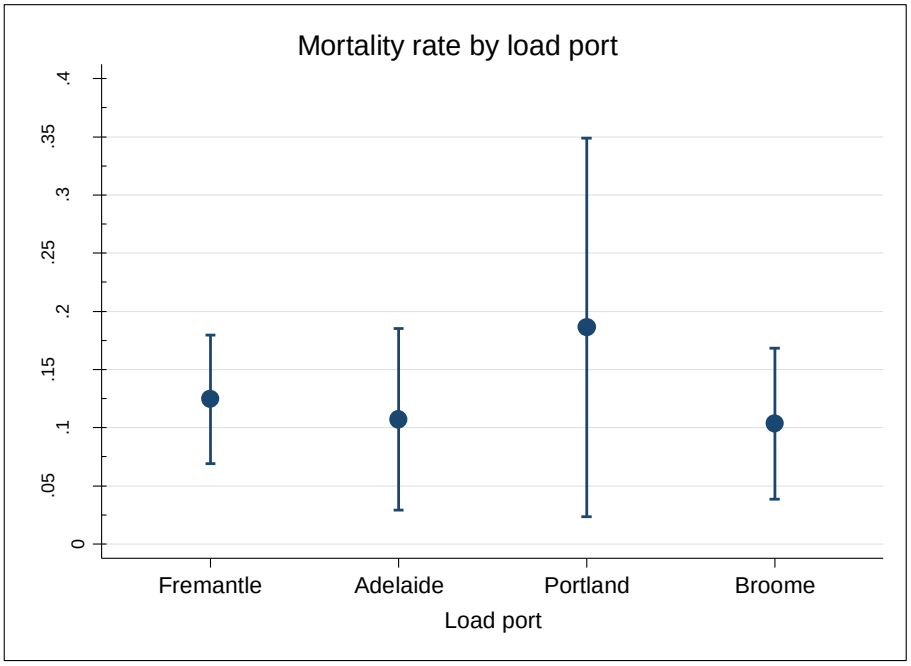

Figure 4: Mean voyage-level mortality rate arranged by load port. Bars represent 95% confidence intervals.

Participating voyages unloaded cattle in multiple countries, which were in turn assigned to one of three broad destination groups: Middle East (Bahrain, Egypt, Israel, Libya and Saudi Arabia), China, and Russia & Turkey (including voyages to the Russian Federation and to Turkey). Figure 5 shows the mean voyage mortality rate by destination country.

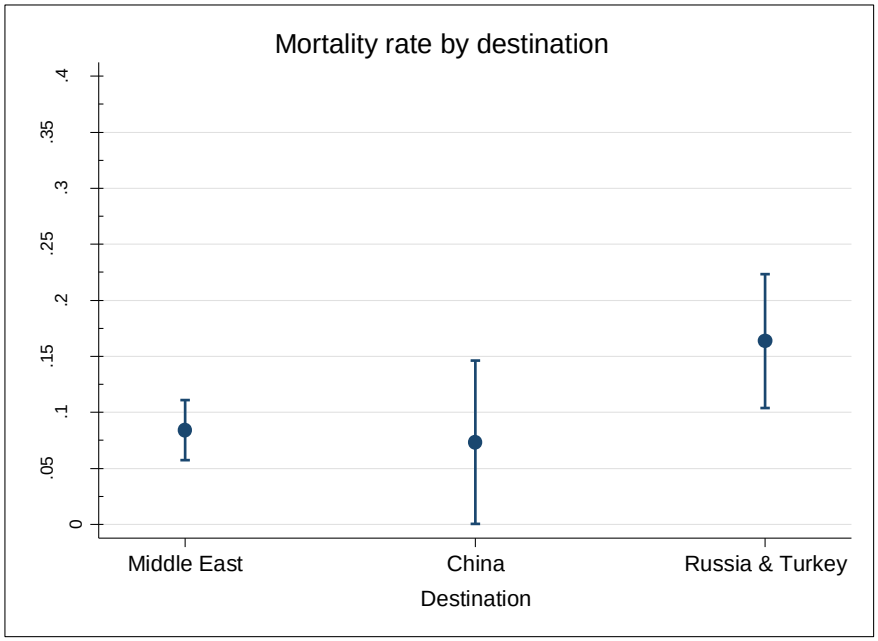

Figure 5: Mean voyage mortality rate arranged by destination country. Bars represent 95% confidence interval.

There was only a single voyage to China and this explains why the confidence interval for mortality rate for this voyage is relatively wide. The voyage mortality rate for voyages to the Middle East (0.08 deaths per 1,000 cattle-days; 95% CI from 0.06 to 0.11) was significantly lower than the voyage mortality rate for voyages to Russia & Turkey (0.16 deaths per 1,000 cattle-days; 95% CI from 0.1 to 0.22; p=0.007). Other comparisons were not significant.

Participating voyages took place over three consecutive years (2010, 2011, 2012). Figure 6 shows the mean voyage mortality rate by year.

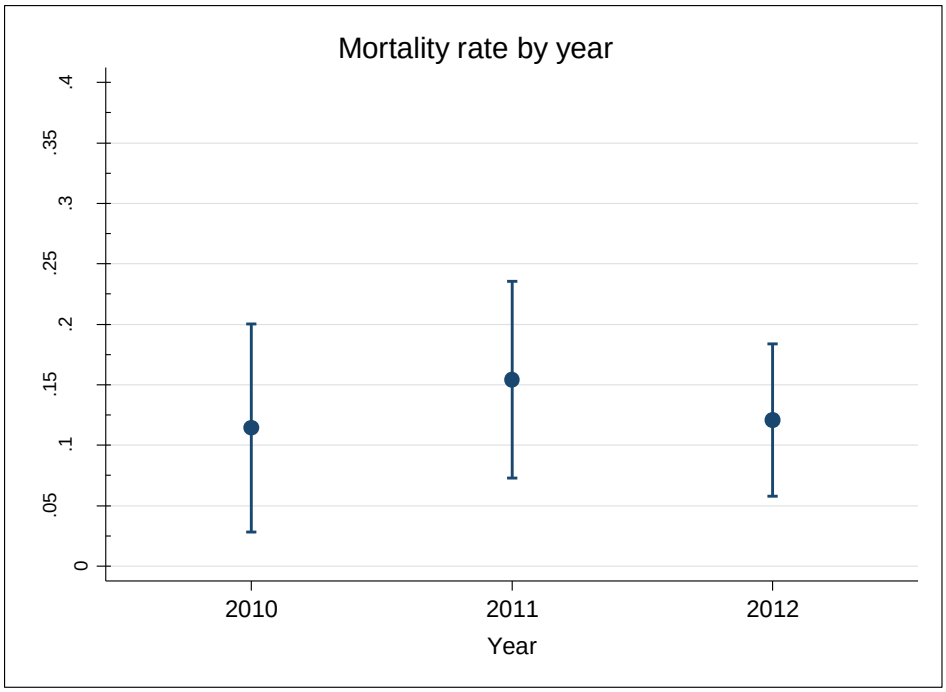

Figure 6: Mean voyage mortality rate arranged by year. Bars represent 95% confidence interval.

There appeared to be some variation in voyage mortality rate from year to year, however the differences were not statistically significant (p>0.05).

7.4 Mortalities that contributed data to the project

Necropsy reports were received from a total of 215 of the 742 mortalities (29%) that were recorded for the participating voyages. This group of 215 animals formed the study population that was analysed in more detail to describe the causes of death in cattle on participating voyages.

Of these 215 animals, 135 (63%) were found dead, 57 were euthanased (26%) and the manner of death was not recorded for the remaining 23 (11%).

The protocol outlined in the Veterinary Export Handbook (W.LIV.0252) asked that AAVs complete a Necropsy/Dead Cattle Report for all dead cattle, as a way of recording deaths.

The protocol then outlined an approach to necropsy that was a compromise intended to reduce time commitment from AAVs by minimising the number of necropsies that were performed while attempting to ensure sufficient necropsies were done to allow characterisation of the major causes of death. The approach involved doing necropsies on three representative animals within a period of several days when multiple deaths occurred with the same or similar presenting signs/syndrome. After that, if additional deaths occurred with the same or similar signs, then the AAV was asked to conduct additional necropsies at a rate of one every 1-2 days from the group of animals dying with similar signs.

It was recognised in the design phase of this project that not every death would be able to be necropsied. The aim was to have necropsies completed on sufficient numbers of dead animals to allow characterisation of the major causes of death.

The results clearly indicate that AAVs were completing forms mainly for those animals that were necropsied and not for all deaths. Information on total numbers of deaths for each voyage was then sourced from other data sources for analysis including voyage mortality reports tabled in parliament and available from the Department of Agriculture website and the Shipboard Mortality Database.

7.5 Clinical presentation

Clinical signs recorded by the AAVs were reviewed and grouped into categories for reporting and analysis (Table 5).

Table 5: Summary of the clinical category recorded for 215 cattle dying on participating voyages

Clinical category	Manner of death			Total	Row total as % of grand total
	Found dead	Euthanased	Not recorded		
Lameness	10	19	2	31	14.4
Recumbency/Weakness	8	20	3	31	14.4
Ill-thrift/Shy feeder	16	5	2	23	10.7
Respiratory signs	11	2	2	15	7.0
Neurological signs	3	7	0	10	4.7
Misadventure	5	3	0	8	3.7
Sudden death	1	0	0	1	0.5
Urinary signs	1	0	0	1	0.5
Pink eye	0	1	0	1	0.5
No previous clinical signs	79	0	0	79	36.7
No clinical category recorded	1	0	14	15	7.0
Grand totals	135	57	23	215	100.0

Of those animals that were found dead, most (n=79) were recorded as having shown no previous clinical signs. This is likely to reflect difficulties associated with intermittent observations of pens across the entire ship rather than evidence that most animals truly show no clinical signs prior to death.

Of the remaining 56 animals, the most common clinical signs recorded prior to death were ill-thrift and/or shy feeders (n=16), respiratory signs (n=11), lameness (n=10), and recumbency and/or weakness (n=8).

The five animals classified as ‘misadventure’ were found dead with their head stuck under a pen rail, and were assumed to have died from circulatory collapse and asphyxiation as a result of being cast.

Four of the animals classified as ‘lameness’ had been sedated for treatment of the lameness and had failed to recover from the anaesthetic.

Fifty-seven animals were euthanased, with the most common reasons being for recumbency and/or weakness (n=20) and for lameness that was not responsive to treatment (n=19). Seven animals were recorded as having neurological signs prior to euthanasia and two with respiratory signs that were not responsive to treatment. Of the three animals euthanased due to misadventure, one went down in the race during discharge and would not stand up again, one was found trapped in a gate and the other was found with severe preputial bruising.

When all records were combined, the major identified clinical categories recorded for mortalities (in order from most to least important) were lameness, recumbency and/or weakness, ill-thrift / shy feeder, respiratory signs, neurologic signs and misadventure.

7.6 Gross pathology

Necropsies were performed on 197 of the 742 deaths (27%) that occurred on the study voyages, representing 92% of the 215 animals that formed the study population.

Core body temperature (CBT) was measured using a deep probe thermometer in 97 animals across 14 voyages. Twelve animals were found to be hyperthermic (CBT ≥ 43°C) but only 2 animals were assigned a final diagnosis of primary hyperthermia (i.e. heat stroke). Hyperthermia secondary to moderate-to-severe respiratory disease was identified in a further 9 animals and one animal with hyperthermia was found dead with no previous clinical signs and no evidence of either heat stroke or respiratory disease. There was a significant association between CBT and time since death ($P < 0.001$) and this was found to be due to a drop in CBT that appeared at between 14 and 16 hr after death (Table 6).

Table 6: Summary of count of gross necropsy diagnosis categories derived from necropsy forms for the 215 deaths forming the study population

Gross necropsy diagnosis	Count	% of total
Respiratory disease	100	46.5
Inflammation of joints/muscle	20	9.3
Inappetence	15	7.0
Misadventure	13	6.0
Enteric disease	10	4.7
Anaesthetic complication	4	1.9
Metabolic disease	4	1.9
Cardiovascular disease	2	0.9
Urinary tract disease	2	0.9
Encephalitis	1	0.5
Neurogenic lameness	1	0.5
No significant lesions	22	10.2
Not recorded	21	9.8
Total	215	100.0

Respiratory disease had been recorded as a clinical sign or syndrome in 15 animals prior to death (Table 5) and 12 of these animals (80% of the 15) had gross pathology indicative of respiratory disease. Two of the 15 animals (13.3%) were found at necropsy to have

cardiovascular disease – one with traumatic reticuloperitonitis (hardware disease) and one with severe restrictive pericarditis. One animal had an impacted abomasum.

Respiratory disease was also a common necropsy diagnosis for animals recorded in other categories of clinical signs/syndromes:

- Of 79 animals recorded with no previous clinical signs, 54 (68%) had respiratory disease at necropsy;
- Of 23 animals recorded with a clinical category of ill-thrift and/or shy feeders, 13 (57%) had respiratory disease at necropsy;
- Of 31 animals recorded with a clinical category of weakness and/or recumbency, 13 (42%) had respiratory disease at necropsy;
- Of 31 animals recorded with a clinical category of lameness, 3 (10%) had respiratory disease at necropsy;
- Of eight animals recorded with misadventure, 1 (12.5%) had respiratory disease at necropsy;
- Respiratory disease was recorded as a gross necropsy diagnosis in a further four animals including two animals that did not have any clinical category recorded, one animal with a clinical category of neurologic disease and one animal with a clinical category of sudden death.

The necropsy form included a diagrammatic layout of the respiratory system to allow AAVs to record the distribution of lung lesions observed at gross necropsy. This information was recorded for 157 of the 197 animals on which a gross necropsy was performed.

Of the 157 animals with information recorded on lung lesions, there were 35 (22% of 157) with no significant lung lesions and 122 (78%) that had lung lesions.

Of the 122 animals with lung lesions that had information recorded on the necropsy form about lung lesion presence and distribution, the following summary information was available about lesions:

- 85 animals (69.7% of 122) had diffuse discoloration and/or consolidation in the cranioventral lobes only;
- 25 animals (20.5% of 122) had diffuse discoloration and/or consolidation in all visible lung;
- Eight animals (6.6% of 122) had multifocal lesions across the lungs;
- Three animals (3.5% of the 122) had focal lesions in specific parts of the lungs; and
- One animal showed both cranioventral and multifocal lesions.

There were 20 animals with a gross necropsy diagnosis of joint/muscle inflammation. These included animals with arthritis, lameness and cellulitis involving musculoskeletal structures. A small number of these animals showed gross necropsy evidence of concurrent diseases including more generalised septicaemia and respiratory disease.

There were 15 animals with a gross necropsy diagnosis of inappetence. Ten of these animals showed evidence of fatty liver at gross necropsy that was suggestive of energy metabolism disorders such as ketosis. It is noteworthy that all ten animals with a gross diagnosis of fatty liver and suspected ketosis were part of a shipment of pregnant, breeding animals (dairy and beef) on the same voyage and nine of these animals were noted as being

pregnant (without details on stage of pregnancy). A small number of these animals showed gross necropsy evidence of concurrent diseases including more generalised septicaemia and respiratory disease.

There were 13 animals with a gross necropsy diagnosis of misadventure. A number of these animals had suffered from inadvertent or accidental deaths from circumstances such as being caught in a rail or gate or trampled in a race. A number of animals showed gross necropsy evidence of concurrent diseases including myocarditis, rumenitis, liver disease and respiratory disease.

There were 10 animals with a gross necropsy diagnosis of enteric disease. These included animals with gross necropsy records of gastrointestinal tract obstruction, enteritis, peritonitis, rumen indigestion, bloat and enterotoxaemia.

The 4 animals with a gross necropsy diagnosis of anaesthetic complications had all been sedated in order to allow a more detailed inspection and treatment of limb conditions associated with lameness and the animals failed to recover successfully from the anaesthetic. Deaths were attributed to complications arising from anaesthesia in compromised animals.

There were 4 animals with a gross necropsy diagnosis of metabolic disease. All of these animals appeared to show varying levels of gait abnormalities and tetanic spasms that were considered suggestive of hypomagnesaemia. Necropsy changes on these animals were relatively unremarkable.

There were 2 animals with a gross necropsy diagnosis of cardiovascular disease. One of these animals was diagnosed with traumatic reticuloperitonitis (hardware disease) at gross necropsy and the other animal showed gross necropsy evidence of pericarditis (inflammation of the sac enclosing the heart and often involving the outer surface of the heart itself).

One animal with a gross necropsy diagnosis of encephalitis had been noticed with neurological signs prior to death.

One animal with a gross necropsy diagnosis of neurogenic lameness had been euthanased as a result of chronic lameness with muscle wasting over the shoulder that was not responding to treatment.

There were 22 animals with a gross necropsy diagnosis of no significant lesions and a further 21 animals that were necropsied (and had samples collected from tissues), but no report or record of necropsy findings was completed.

7.6.1 Association between gross necropsy findings and clinical category record

There was general agreement in many cases between the information recorded about clinical signs or syndromes displayed by animals before death or euthanasia. However, there were a number of cases where gross necropsy findings indicated pathology in organ systems other than those that might be considered to be most directly related to the clinical category record for that animal.

These occurrences were not unexpected. It can be difficult to observe all clinical signs in animals suffering from one or multiple disease conditions during a voyage. Many animals

may be compromised by one initial condition that may result in increased susceptibility to a variety of additional conditions and then develop additional secondary diseases. An initial inciting event may then be obscured by secondary or opportunistic diseases that may in turn develop into more serious and potentially life threatening diseases.

This section reviews the records of animals by clinical category and provides a brief summary of the gross necropsy findings within each category of clinical record. Some of this information will be repeating what has been described previously in the summary of gross necropsy findings but in a different presentation order. This is considered justified because this information does shed light on the range of necropsy findings in animals with various clinical syndromes.

There were 31 animals with a clinical syndrome recorded of lameness. The diagnoses recorded for these animals at gross necropsy included:

- Seventeen of the 31 (55%) had a gross necropsy diagnosis of inflammation of joints and/or muscle;
- Four of the 31 (13%) were sedated to treat a musculoskeletal condition and subsequently did not recover from the anaesthetic;
- Three of the 31 (10%) had respiratory disease;
- Two of the 31 (6%) had broken legs and were euthanased;
- Two of the 31 (6%) had neurogenic lameness with muscle wasting and weakness and were euthanased; and
- Three animals had gross necropsy diagnoses of no lesion (n=1), no necropsy performed (n=1) and metabolic disease (n=1).

There were 31 animals with a clinical syndrome recorded of recumbency or weakness. The diagnoses recorded for these animals at gross necropsy included:

- Thirteen of the 31 (39%) had a gross necropsy diagnosis of respiratory disease;
- Eleven of the 31 (35%) had a gross necropsy diagnosis of no significant lesion;
- Five of the 31 (16%) had a gross necropsy diagnosis of inappetence and two of these were noted to have fatty liver; and
- Two animals had a gross necropsy diagnosis of enteric disease (n=1) and urinary tract disease (n=1).

There were 23 animals that had a clinical syndrome of ill thrift or shy feeder recorded. The diagnoses recorded for these animals at gross necropsy included:

- Seven of the 23 (30%) had a gross necropsy diagnosis of inappetence and five of these animals were recorded with fatty liver;
- Thirteen of the 23 (52%) had a gross necropsy diagnosis of respiratory disease;
- Two animals (9%) had a gross necropsy diagnosis of no significant or no recorded lesions; and
- One animal had a gross necropsy diagnosis of enteric disease.

There were 10 animals that had a clinical syndrome of neurologic disease recorded. The diagnoses recorded for these animals at gross necropsy included:

- Three of the 10 (30%) had a gross necropsy diagnosis of metabolic disease with a note of suspected hypomagnesaemia;
- Two of the 10 (20%) had a gross necropsy diagnosis of enteric disease;
- Two of the 10 (20%) had a gross necropsy diagnosis of no significant lesion; and,
- Three animals had a gross necropsy diagnosis of encephalitis (n=1), misadventure / trauma (n=1) and respiratory disease (n=1).

There were eight animals that had a clinical syndrome of misadventure recorded. The diagnoses recorded for these animals at gross necropsy included:

- Six of the 8 (75%) had a gross necropsy diagnosis of misadventure including animals that had been trapped in a rail or gate, trampled in a race and miscellaneous trauma leading to severe preputial bruising;
- One animal (12.5%) had a gross necropsy diagnosis of respiratory disease; and.
- One animal (12.5%) had a gross necropsy diagnosis of no lesion.

7.7 Histology

Histology findings were not available until collected samples had been imported back into Australia, processed and examined by pathologists working as part of the project team. For those voyages where a ship returned to Fremantle directly after completing one voyage to begin another voyage, samples were collected within weeks of the end of one voyage. There were occasions where a ship completed a voyage as part of this project and then sailed to another port either in Australia or elsewhere in the world. In a small number of cases there was a delay of some months from the end of a voyage until the ship docked in Fremantle again and samples from the participating voyage could be unloaded and processed for examination.

Between 1 and 16 tissues were collected from animals at necropsy, with an average of 6 ± 3 tissues per animal. The most commonly collected tissues were lung (collected from 99% of necropsies), heart (80%), kidney (74%), liver (73%), trachea (67.5%), and rumen (46.2%).

Collected tissue samples were labelled and stored in formalin on the ship for the return journey and were collected when the ship docked at Fremantle. Samples were transported to the DAFWA laboratory and processed for routine microscopic examination to describe presence or absence of pathologic changes in tissues.

Abnormal or pathologic changes were detected in about 40% of tissue samples.

Approximately 50% of tissues were histologically normal and the remaining samples were unsuitable for examination mainly because the tissues were too autolysed to allow effective examination and in some cases, because the tissues were not relevant to the clinical category or gross necropsy changes for that animal.

Rapid onset of autolysis of internal organs is a common issue during the voyage because of environmental conditions. In addition there was usually a time delay between death and necropsy and in some cases this delay was exacerbated because necropsies were being performed by a single AAV in conjunction with other routine tasks.

The lung was collected for histological examination from 195 out of 197 (99%) necropsies. Histologic examination of the 195 lung tissue samples resulted in the following findings:

A total of 127 of the 195 animals (65%) had histologic evidence of respiratory disease based on examination of lung tissue.

Of the 127 animals with histologic evidence of lung disease, the most common pathology observed was bronchopneumonia, which was present in 82% of cases. In some cases bronchopneumonia was observed in combination with pleuritis. Interstitial pneumonia was observed far less commonly (6%) in animals with histologic evidence of respiratory disease. There were occasional cases of pleuritis without lung tissue involvement and tracheitis.

> ***Pneumonia*** *means inflammation of the functional tissues of the lungs;* ***bronchopneumonia*** *means inflammation of the lungs and of the larger airways (bronchioles);* ***pleuritis*** *or* ***pleuropneumonia*** *means the inflammation has extended to the outer pleural surface of the lung;* ***interstitial pneumonia*** *means inflammation of the functional tissue of the lungs without inflammation of the bronchioles;* ***tracheitis*** *means inflammation of the trachea.*

Descriptions of histologic lesions in lung tissue – including morphology of intralesional bacteria where present – were used to identify the viral and bacterial pathogens considered most likely to be causing the infection. This approach was based in part on extensive scientific descriptions of the most common pathogens (viruses and bacteria) that cause respiratory disease in cattle and the typical histologic changes in lung tissue in animals with respiratory disease known to be caused by infection with specific pathogens.

The common and most important bacterial pathogens associated with respiratory disease in cattle include: *Histophilus somni, Mannheimia haemolytica, Pasteurella multocida* and *Mycoplasma bovis*.

The common and most important viral pathogens and their abbreviated names include:

- BCoV : Bovine corona virus;
- BoHV-1 : Bovine herpes virus 1;
- BRSV : Bovine respiratory syncytial virus;
- BVDV : Bovine viral diarrhoea virus; and
- BPIV-3 : Bovine parainfluenza virus 3.

Histologic evidence of bacterial infection in association with pneumonia lesions was present in 92 of 102 lung tissue samples from animals with pneumonia (90%). Five samples had histologic changes that were consistent with primary viral infection (5% of 102). Five samples had equivocal histologic changes that did not allow characterisation of the likely infectious agent(s).

Of the 92 pneumonia cases with histologic evidence suggestive of bacterial infection:

- 46 of 92 (50%) had changes that were suggestive of bacterial infection without specifically suggesting any one of the four bacteria of interest;
- 26 of 92 (28%) had changes that were suggestive of *Mycoplasma bovis* infection in combination with one or more of the other three bacterial agents;

- 14 of 92 (15%) had changes that were suggestive of *Mycoplasma bovis* infection as a primary cause of pneumonia; and,
- 6 of the 92 (7%) had changes that were suggestive of bacterial infection in combination with viral infection.

A large body of descriptive information was generated on histologic changes in tissue samples collected during the project. The information was used in conjunction with all other available information relevant to disease before death, at necropsy and in pathologic examination of tissues or swabs to determine the most likely cause of death. Further descriptions of histologic changes are not provided here because the range of changes presented in isolation is likely to be relatively uninformative.

In some cases histologic evidence in other tissues was noteworthy because it identified unusual potential diseases or was suggestive of specific pathogens of interest.

For example, there were 16 cases where examination of heart tissue identified pathology involving the heart. These included 10 animals with myocarditis lesions and in these 10 animals histologic changes were suggestive of specific conditions:

- Acute myocarditis lesions were observed in five animals and in three of these there were concurrent lesions in other tissues that were considered consistent with systemic infection with *Histophilus somni*, also called *Haemophilus* septicaemia or *Haemophilus somnus* disease complex; and
- Chronic myocarditis lesions were observed in five animals and three of these had sarcocysts present in the heart muscle, indicating infection with *Sarcocystis* species of sporozoan parasite.

7.8 Molecular results

Molecular analyses involved the application of specific quantitative polymerase chain reaction (qPCR) assays to lung tissue and swabs (nasal swabs or lung tissue swabs) to detect the presence of genetic material indicative of respiratory disease pathogens including the four bacterial pathogens and five viral pathogens of particular interest for this project.

Presence alone of the infectious agents of interest based on qPCR tests is difficult to interpret, because healthy animals may carry these agents without necessarily suffering from disease. A decision was made to examine qPCR results in conjunction with results from histologic examination of tissue samples from the same animals in order to allow better interpretation of the qPCR results in light of the presence or absence of lung disease based on histologic examination of lung tissue samples.

Samples suitable for qPCR assay were collected from 159 animals but eight of these animals did not have matched lung tissue samples for histology examination.

There were 151 animals that had matched lung and/or nasal samples allowing qPCR tests for viral agents to be completed and compared to histology findings from lung tissues collected on the same animals.

There were 128 animals that had matched lung and/or nasal samples allowing qPCR tests for bacterial agents to be completed and compared to histology findings from lung tissues collected from the same animals.

Results are presented in Table 7.

For the 151 samples that had both viral qPCR tests and histology, the histology results were used to classify animals as either having pneumonia (n=102) or not having pneumonia (n=49).

A qPCR positive test result indicates presence of genetic material in the sample from the specific pathogen being tested. The presence of a qPCR positive test result does not necessarily mean that the tissue contained live, active infective material from the specific pathogen at the time the animal died. It means that at some time in the period before the animal died the specific pathogen almost certainly had been present in that tissue. In some cases even after an animal has been infected and recovered there may still be small amounts of residual genetic material present in tissues that is no longer viable but that may still be detected using a qPCR test. In this report we are classifying a qPCR positive test result as indicating that the animal had recently been infected with that pathogen.

Results from the qPCR tests detecting presence of viral genetic material can then be directly compared between those animals with histologic pneumonia (abnormal lung) and those without histologic pneumonia (normal lung). The odds ratio provides a statistical test to compare the strength of association between qPCR status (positive vs negative) and histologic status (pneumonia vs no pneumonia). When the odds ratio is greater than one it is indicating that animals with pneumonia were more likely to have positive qPCR results compared to animals without pneumonia. When the odds ratio is less than one it is indicating that animals with pneumonia were less likely to have positive qPCR results compared to animals without pneumonia. The p-value and confidence intervals can be used to determine if the odds ratio is statistically significant. If the p-value is less than 0.05 or if the confidence interval for the odds ratio does not include one, then the comparison is significant.

The prevalence of the viruses of interest in lung samples was relatively low and there was not a lot of difference between the group with histologic pneumonia and the group without histologic pneumonia. In fact none of the statistical comparisons were statistically significant, indicating that qPCR positive test results for viral pathogens were just as likely to occur in animals without pneumonia as they were in animals with pneumonia.

There was one comparison that was almost significant, involving Bovine Viral Diarrhoea Virus (BVDV). Animals with histologic pneumonia had a 4.1-fold higher odds of being qPCR positive for BVDV compared to animals that had no histologic evidence of pneumonia. The p-value for this comparison was 0.053. All other comparisons were not significant ($p>0.05$).

The prevalence of bacterial pathogens in animals with histologic pneumonia ranged from 35 to 60% and was significantly higher than the presence of the same bacteria in samples from animals with no histologic evidence of pneumonia (prevalence ranging from 2 to 14%).

For each specific bacterial pathogen, the statistical comparisons were highly significant (Table 7):

- Animals with histologic pneumonia had a 4.1-fold higher odds of being qPCR positive for *H. somni* compared to animals that had no histologic evidence of pneumonia ($p<0.001$);
- Animals with histologic pneumonia had a 63.2-fold higher odds of being qPCR positive for *M. bovis* compared to animals that had no histologic evidence of pneumonia ($p<0.001$);
- Animals with histologic pneumonia had a 11.1-fold higher odds of being qPCR positive for *M. haemolytica* compared to animals that had no histologic evidence of pneumonia ($p<0.001$); and
- Animals with histologic pneumonia had a 9.3-fold higher odds of being qPCR positive for *P. multocida* compared to animals that had no histologic evidence of pneumonia ($p<0.001$).

In the previous section of this report, histologic changes in lung tissues from those animals with pneumonia were used to tentatively identify specific pathogens (bacteria and/or viruses) that were considered likely to have been involved in the underlying pneumonia. These results were then compared to the findings from qPCR detection of pathogens in lung samples.

Where histology results indicated that bacterial infection was likely to have been involved in the pneumonia, qPCR tests were positive for one or more of the bacterial agents in 75% of cases.

Where histology results indicated that viral infection was likely to have been involved in the pneumonia, qPCR results were positive for one or more viral pathogens in 45% of cases.

There were a number of cases where multiple pathogens were present in the same tissue samples indicating concurrent infection with more than one pathogen. Simple correlations were performed as a crude measure of whether some combinations of pathogens were more likely to have occurred together than as a result of chance alone.

The presence of *H. somni* in lung tissue was significantly correlated with the presence of *M. bovis, P. multocida*, and viral pathogens ($p<0.001$), in particular BCoV and BVDV ($p< 0.01$). The presence of *M. bovis* and *M. haemolytica* were significantly correlated with the presence of *P. multocida* ($p<0.001$).

Table 7: Summary of results of qPCR detection of specific genetic material in lung samples for viral pathogens from 151 animals and for bacterial pathogens from 128 animals. Each row provides a count of the number of animals tested from two groups (with and without histologic evidence of pneumonia), the number of animals with qPCR positive test results for each pathogen, and the odds ratio comparing the odds of qPCR positive results in animals with histologic pneumonia to the odds of being qPCR positive in animals without histologic pneumonia. OR=odds ratio, CI=confidence interval.

Pathogens detected in lung samples	Animals with histologic evidence of pneumonia			Animals with no histologic evidence of pneumonia			Odds ratio	95% CI for OR	p-value
	qPCR + (n)	No. tested (N)	Prevalence (% of N)	qPCR + (n)	No. tested (N)	Prevalence (% of N)	(OR)		
Viruses									
BCoV	11	102	10.8	4	49	8.2	1.4	0.4 to 6.2	0.6
BoHV-1	3	102	2.9	0	49	0.0	2	0.2 to 100.4	0.5
BRSV	2	102	2.0	4	49	8.2	0.23	0.02 to 1.7	0.07
BVDV	15	102	14.7	2	49	4.1	4.1	0.9 to 37.7	0.053
BPIV-3	0	102	0.0	0	49	0.0	0.5	0.01 to 38.9	0.6
Bacteria									
H. somni	40	84	47.6	6	44	13.6	5.8	2.1 to 18.2	<0.001
M. bovis	50	84	59.5	1	44	2.3	63.2	9.5 to 2604.2	<0.001
M. haemolytica	29	84	34.5	2	44	4.5	11.1	2.5 to 99.6	<0.001
P. multocida	34	84	40.5	3	44	6.8	9.3	2.6 to 49.9	<0.001

Nasal swabs were collected at necropsy from 84 animals.

Matched nasal swab samples and lung histology results were available for 82 animals.

Histology findings from microscopic examination of lung tissue samples were used to classify the 82 animals into those animals with histologic evidence of pneumonia (n=57) and those animals with no histologic evidence of pneumonia (n=25).

The qPCR tests of nasal swabs from the same animals were used to classify animals as positive or negative for each specific viral and bacterial pathogen.

The prevalence for each pathogen in animals with histologic pneumonia was then compared to the prevalence for each pathogen in animals with no histologic evidence of pneumonia. A statistical test based on the odds ratio was used to test whether animals with histologic pneumonia were more or less likely to have pathogens detected in nasal swabs.

Results are presented in Table 8.

Respiratory viruses were detected in nasal swabs of animals with and without histologic evidence of pneumonia. The prevalence of qPCR positive results from nasal swabs in animals with histologic pneumonia appeared numerically higher than the prevalence in nasal swabs from animals with no histologic evidence of pneumonia but statistical comparisons of these associations returned non-significant results for all the viruses that were tested ($p<0.05$). The results indicate that the presence of a viral pathogen in a nasal swab has little relationship with the likelihood of that animal having histologic evidence of pneumonia.

Bacterial pathogens were detected in nasal swabs of animals with and without histologic evidence of pneumonia. The prevalence of bacterial pathogens in nasal swabs appeared to be higher in animals with histologic evidence of pneumonia compared to those animals with no histologic evidence of pneumonia. Statistical testing of the association between presence of nasal qPCR positive result and presence or absence of histologic evidence of pneumonia returned significant results for three of the four bacteria tested (Table 8):

- Animals with histologic pneumonia had a 1.7-fold higher odds of being qPCR positive for *H. somni* in nasal swabs compared to animals without pneumonia ($p=0.3$);
- Animals with histologic pneumonia had a 15-fold higher odds of being qPCR positive for *M. bovis* in nasal swabs compared to animals without pneumonia ($p<0.001$);
- Animals with histologic pneumonia had a 7.2-fold higher odds of being qPCR positive for *M. haemolytica* in nasal swabs compared to animals without pneumonia ($p=0.01$); and,
- Animals with histologic pneumonia had a 8.4-fold higher odds of being qPCR positive for *P. multocida* in nasal swabs compared to animals without pneumonia ($p<0.001$).

Table 8: Summary of results of qPCR detection of specific genetic material in nasal swab for pathogens in nasal swabs from 82 animals and for histology findings from lung tissues from the same animals. Each row provides a count of the number of animals tested from two groups (with and without histologic evidence of pneumonia), the number of animals with qPCR positive test results for each pathogen, and the odds ratio comparing the odds of qPCR positive results in animals with histologic pneumonia to the odds of being qPCR positive in animals without histologic pneumonia. OR=odds ratio, CI=confidence interval.

Pathogens detected in nasal swabs	Animals with histologic evidence of pneumonia			Animals with no histologic evidence of pneumonia			Odds ratio	95% CI for OR	p-value
	qPCR + (n)	No. tested (N)	Prevalence (% of N)	qPCR + (n)	No. tested (N)	Prevalence (% of N)	(OR)		
Viruses									
BCoV	9	57	15.8	2	25	8.0	2.2	0.4 to 21.9	0.3
BoHV-1	9	57	15.8	1	25	4.0	4.5	0.6 to 205.3	0.1
BRSV	2	57	3.5	0	25	0.0	1.4	0.1 to 75.9	0.8
BVDV	4	57	7.0	1	25	4.0	1.8	0.2 to 92.9	0.6
BPIV-3	1	57	1.8	0	25	0.0	0.9	0.05 to 56.9	0.9
Bacteria									
H. somni	30	57	52.6	10	25	40.0	1.7	0.6 to 4.9	0.3
M. bovis	34	57	59.6	2	25	8.0	15	3.1 to 139	<0.001
M. haemolytica	22	57	38.6	2	25	8.0	7.2	1.5 to 67.9	0.01
P. multocida	35	57	61.4	4	25	16.0	8.4	2.3 to 36.9	<0.001

There were 79 animals that had qPCR test results available from both nasal swab samples and from lung samples.

Results are presented in Table 9.

The results suggest that detection of a pathogen in a nasal swab is associated with an increased risk of that same pathogen being detected in lung tissue and that this risk varies between pathogens.

Caution is urged in interpreting these findings because of the low number of samples in some combinations. The sparse sample size in some cells is reflected in the wide confidence intervals for odds ratio estimates.

However, the results do suggest that nasal detection of pathogens may be a marker of risk for presence of the same pathogens in lung. Results presented earlier in this section indicated that there is an association between detection of a pathogen in lung tissue and the presence of pneumonia.

Table 9: Summary of results of qPCR detection of viruses and bacteria in matched samples from nasal swabs and lung tissue. Odds ratios are presented as a measure of the association between the odds of returning a positive lung test in animals with a positive nose test compared to the odds of returning a positive lung test in those animals with a negative nose test.

		qPCR results for each combination of nose and lung samples								
Pathogens	**No. of animals tested** (N)	**Nose + Lung - (n)**	**Nose + Lung + (n)**	**Lung + as % of all nose + (%)**	**Nose - Lung + (n)**	**Nose - Lung - (n)**	**Lung + as % of all nose - (%)**	**Odds ratio (OR)**	**95% CI for OR**	**p-value**
Viruses										
BCoV	79	7	4	36.4	2	66	2.9	18.9	2.1 to 226	0.003
BoHV-1	79	7	3	30.0	0	69	0.0	34.5	2.7 to 1721	0.001
BRSV	79	1	1	50.0	1	76	1.3	76	0.5 to 6024	0.05
BVDV	79	2	3	60.0	2	72	2.7	54	3.4 to 871	0.001
BPIV-3	79	1	0	0.0	0	78	0.0	39.5	0.3 to 3132	0.07
Bacteria										
H. somni	79	18	21	53.8	4	36	10.0	10.5	2.9 to 46.9	<0.001
M. bovis	79	11	23	67.6	5	40	11.1	16.7	4.6 to 66.8	<0.001
M. haemolytica	79	14	9	39.1	7	49	12.5	4.5	1.2 to 16.8	0.01
P. multocida	79	20	17	45.9	3	39	7.1	11.1	2.7 to 63.7	<0.001

Swabs were collected from a small number of animals presenting with diseases other than pneumonia.

There were nine swabs collected from diseased joints or from peripheral cellulitis lesions. Of these samples two (22%) returned a positive qPCR test for *Mycoplasma bovis (M. bovis)*.

There were a further five animals that had returned a positive qPCR test result for *Mycoplasma bovis* on lung and/or nasal swabs and that were also noted as having been lame in the clinical category record. None of these animals had swabs collected from the site of the lameness so it was not possible to explore qPCR testing to look for presence of *M. bovis* or any other pathogen in musculoskeletal lesions.

7.9 Final diagnosis on cause of death

Once all investigations were concluded, the 215 animals comprising the study population were assigned to one of eight categories of causes of death:

- Respiratory disease;
- Musculoskeletal conditions and injuries;
    - included lameness, misadventure and four cases of anaesthetic complication where animals were anaesthetised to treat underlying lameness conditions
- Ketosis;
- Septicaemia;
- Enteric disease;
- Heat stroke; and
- Other.

The materials and methods section described the pathologic findings used to assign animals to final causes of death for respiratory disease and heat stroke in particular. These criteria were defined prior to the start of the project in anticipation that these causes would be likely to be important.

Other final causes of death were based on the combination of all information available including clinical category, necropsy findings, and pathology and laboratory examinations performed on samples imported back into Australia. Where there were additional clinical notes or history on individual animals and in some cases digital photographs these were added to the pool of information used to assign animals to a final cause of death.

Some of the categories for final cause of death are reasonably self-explanatory and can be understood fairly easily particularly once the report is read with additional descriptive information about animals in each category.

Some brief technical descriptions are provided here for ketosis, septicaemia and enteric disease in order to ensure the reader has an understanding of these terms.

Ketosis is literally a state of elevated levels of ketones in the body. Ketosis is a syndrome of disrupted energy metabolism in cattle and is more likely to occur in cattle with elevated energy requirements (pregnant or lactating animals) and with interrupted or inadequate energy intake associated with low blood sugar levels. In non-pregnant and non-lactating cattle on export voyages ketosis may occur as a result of inadequate feed intake often

associated with some form of increased energy requirement resulting from stress and/or concurrent disease. Ketosis may therefore occur as a result of shy feeders or inappetence and secondary to diseases that may suppress appetite and increase metabolic demand in association with infection for example. When these conditions occur the physiologic response is for the liver to increase production of sugars from the metabolism of body fat and to a lesser extent protein. A side effect of this activity within the liver is the production of ketones and in some cases the deposition of increased levels of fat within liver tissue (fatty liver). Pathology and clinical signs develop secondary to a combination of prolonged energy insufficiency (low blood sugar), direct toxicity to the body due to excessive accumulation of ketones which are poisonous in high levels and chronic liver dysfunction secondary to fatty liver changes. Animals may show signs of weight loss, reduced appetite and progressive lethargy (low sugar) while ketosis may produce unusual behavioural changes and neurologic signs.

Septicaemia is the acute invasion of the systemic circulation by pathogenic bacteria resulting in widespread bacterial dissemination and infection involving multiple organs and tissues throughout the body, and culminating with septic shock and an elevated risk of rapid death if untreated. Bacterial pathogens causing septicaemia produce toxins that result in many of the acute signs of toxic shock and much of the rapid elevation in mortality risk. Acute septicaemia is often associated with relatively non-localised or systemic signs of shock (fever, depression, cardiovascular collapse). Animals that survive acute sepsis may then incur bacterial infection localising in one or more of the body systems or organs that may produce more localised clinical signs, depending on which organs or tissues are most affected by the localising infection (heart, joints, brain, eyes, etc).

The term ***enteric disease*** is a descriptive term that includes a range of diseases involving the gastrointestinal tract. A literal definition of enteric means *relating to the intestines*. In this usage we are broadening the term to apply to the gastrointestinal tract from rumen through to large intestine. Examples of specific conditions included in this broad category include vagus indigestion, bloat, enteritis (inflammation of the intestinal mucosa often associated with diarrhoea), intestinal obstruction, peritonitis and abomasal displacement.

Table 10 shows summary information on the number of cases assigned to each of the major pathologic categories of cause of death.

Table 10: Summary of final pathologic diagnosis for cause of death for the 215 mortalities included in the study population. Causes are ranked in descending order of importance and the final column provides accumulative percentage of the total mortality. CI = Confidence Interval

	Count	Mortality as a %	95% CI		Cum % of
Cause of death	**n**	**% of Total**	**Lower**	**Upper**	**total**
Respiratory disease	107	49.8	40.8	60.1	49.8
Musculoskeletal / injury	33	15.3	10.6	21.6	65.1
Ketosis (inappetence)	12	5.6	2.9	9.7	70.7
Septicemia	11	5.1	2.6	9.2	75.8
Enteric disease	10	4.7	2.2	8.6	80.5
Heat stroke	2	0.9	0.1	3.4	81.4
Other	6	2.8	1	6.1	84.2
No diagnosis	34	15.8	11	22.1	100.0
Total	215	100			

Respiratory disease was the most common cause of death, accounting for nearly 50% of the 215 deaths in the study population.

There are a number of interesting issues in considering the information available at different steps along the pathway to a diagnosis, using respiratory disease as the example.

The first is that records based on clinical observations collected while animals were alive during the voyage provided a poor representation of the importance of respiratory disease. The necropsy forms asked the AAV to provide a clinical category record for animals that died where this information was available. The clinical category can be considered as a syndrome description in that it is descriptive, relatively broad and likely to include multiple individual clinical signs and multiple possible diseases. There were only 15 animals from the 215 (7%) recorded with respiratory disease as a clinical category.

At gross necropsy there were 100 animals recorded with a gross necropsy diagnosis of respiratory disease. Once all examinations were completed (including laboratory testing and histology), 90 of these 100 were classified with respiratory disease as the final cause of death.

Ten animals had a gross necropsy diagnosis of respiratory disease and were re-classified to another final cause of death following completion of all examinations. These included:

- four animals with no evidence of lung pathology on histologic examination of lung tissue and that were classified as having a final cause of death of no diagnosis;
- two animals with generalised septicaemia that included pneumonia, however the pneumonia changes were not likely to have resulted in death;
- two animals with heat stroke (one of these had mild to moderate pneumonia that was unlikely to have been serious enough to have caused death);
- one animal with enteric disease and peritonitis; and
- one animal classified as misadventure which had a prior history of being cast and had mild to moderate respiratory disease that was considered unlikely to have resulted in death.

There were 17 animals that were assigned to a final cause of death of respiratory disease that had been recorded with different gross necropsy diagnoses. These included:

- Ten animals that had no necropsy diagnosis recorded;
- Three animals with a gross necropsy diagnosis of misadventure associated with a history of being cast, injured or recumbent;
- Two animals with a gross necropsy diagnosis of inappetence;
- One animal with a gross necropsy diagnosis of inflammation of muscle or joints; and
- One animal with no significant lesions recorded at gross necropsy.

It is also noteworthy that of the 108 animals that had causes of death other than respiratory disease, there was histologic evidence of concurrent respiratory disease in about one third of these animals. Concurrent respiratory disease was observed in animals in every category of final cause of death, with the exception of anaesthetic complication.

A total of 33 animals had a final cause of death of ***musculoskeletal and injury*** conditions.

Of these animals, 24 had lameness recorded as a clinical category. These included four animals that had a gross necropsy cause of death recorded as anaesthetic complication.

These four were recoded into a final cause of death of lameness for this report, because all four had only received anaesthetic in order to have an underlying lameness condition treated.

There were seven cases with a gross necropsy diagnosis of misadventure that were recoded to a final cause of death of musculoskeletal / injury, because all of these animals had traumatic accidents resulting in smothering or trampling as a result of being trapped or cast.

Two additional animals were assigned to lameness as a final cause of death following completion of all pathology examinations. One animal had been found dead with no previous clinical sign recorded and was diagnosed with a septic arthritis and tenosynovitis at necropsy. Another animal was recorded in the clinical category of recumbency / weakness and was reclassified following all other examinations.

There were a number of animals with ante-mortem conditions that appeared to involve the musculoskeletal system, but that were not assigned to a final cause of death of lameness. These included:

Seven animals had lameness recorded as a clinical category and were assigned to other final causes of death following necropsy and completion of all pathology examinations, including:

- Three animals that were assigned to a final cause of death of respiratory disease and that had lameness recorded as an ante-mortem clinical category;
- Two animals that were assigned to a final cause of death of septicaemia where the septicaemia appeared to have been preceded by musculoskeletal signs including lameness and where the animals had been treated but had not responded;
- One animal with a final cause of death of ketosis that had an ante-mortem clinical history of a musculoskeletal wound, a clinical category record of lameness and that subsequently appeared to have become septicaemic, inappetent and recumbent before dying; and
- One animal with a final cause of death of no diagnosis, that had been treated for lameness and appeared to have recovered and was then found dead with no cause of death identified.

Leg wounds and cellulitis lesions were noted in two additional cases assigned to a final cause of death of septicaemia with notes suggesting that the septicaemia may have developed secondary to an initial musculoskeletal injury or infection.

There was also one animal with a final cause of death of septicaemia that had a prior leg wound, which may have contributed to the septicaemia.

Twenty of the 33 animals diagnosed with musculoskeletal / injury conditions were euthanased because their injuries were significantly compromising their welfare.

Of the 33 cases with musculoskeletal / injury conditions as the final cause of death, 20 (61%) occurred on one voyage, raising questions about the possibility of either voyage-associated factors that may contribute to lameness risk (such as sea conditions), ship-associated factors (flooring and pen design), animal factors (riding behaviour), and management factors that may have been different on that voyage in comparison to other voyages.

Other categories of final cause of death have been described in some detail in earlier sections of this report and are briefly summarised here.

There were 12 animals assigned to a final cause of death of ***ketosis***. Ketosis as a cause of death may be considered to be a proxy for inappetence.

Most of the cases of ketosis occurred on a single shipment of pregnant dairy and beef breeder cattle sailing from Portland to the Russian Federation. Suspected predisposing risk factors for death identified by the AAV accompanying the voyage included a 2-week delay from the scheduled sail date, voyage length (31 days), suboptimal access to feed and water, and hot and humid conditions in the Gulf of Aden and Red Sea, followed by cold conditions in the Mediterranean Sea.

Given that most of the ketosis / inappetence cases occurred in pregnant breeder animals, this cause of death may not be as important in feeder or slaughter animals.

The 11 cases of ***septicaemia*** showed multi-organ pathology consistent with widespread infection. Some cases showed a variety of possible inciting causes including respiratory disease, urinary tract infection, musculoskeletal injuries or infections while other cases appeared to have little evidence of an origin or starting point for the septicaemia.

The 10 cases of ***enteric disease*** included cases of traumatic reticuloperitonitis, diarrhoea (enteritis, colitis, enterocolitis), rumenitis, bloat, gastronintestinal tract obstruction and peritonitis.

There were only two cases of confirmed ***heat stroke*** in the 215 mortality cases investigated during this project.

There were six animals with cause of death recorded as ***other***. These included four animals with heart disease, three of which were likely to have been associated with septicaemia associated with *Histophilus somni* infection and one with pericarditis of unknown origin. The other two animals included one with severe pink eye infection and one with a urinary tract infection (pyelonephritis).

A final cause of death could not be determined for 34 of the 215 cases (16%). The number of different tissues sampled at necropsy and whether or not lung tissue in particular was sampled or even whether a completed necropsy form was present, all appeared to have no statistical association with the likelihood of a case being assigned to the category of no diagnosis for final cause of death.

There were 79 animals that had been found dead where the AAV had recorded ***no previous clinical signs*** on the necropsy space where the AAV was asked to provide a clinical category. Of these 79 animals, a gross necropsy diagnosis was recorded for 68 (86%) and a final cause of death was then recorded for all 68 animals.

There were 22 animals (10% of the 215 study cases) that had no significant lesion recorded at gross necropsy (including 5 animals with autolysis that prevented lesions being detected at necropsy). A final cause of death was able to be assigned to 9 animals (41% of 22) after all pathology examinations and tests had been completed.

The following three tables present summary information on classification of the 215 cases of mortality based on clinical category (categories derived from clinical signs recorded by AAV), gross necropsy diagnosis and final cause of death. Each table provides a cross-classification showing agreement between two of the different classification stages.

Table 11 shows the cross classification between clinical category and gross necropsy diagnosis and Table 12 shows the cross classification between clinical category and final cause of death. Table 13 shows the cross classification between findings of gross necropsy and final cause of death.

Clinical category information is a representation of the ante-mortem information that is able to be recorded based on observations from hospital records and pen inspections of animals before they died or were euthanased. There was a relatively large number of animals for which no clinical category information was available (94 of 215 or 44%). This is unsurprising given the relatively large number of animals and pens on export vessels and practical constraints which mean that any individual pen or animal is only likely to be under direct observation for relatively small proportions of time in any day. In addition, cattle standing in a pen will often show little externally visible sign of illness and even if all pens were under constant observation over the pen rail, clinical category information would still be likely to detect some animals with illness.

Clinical category information was poorly indicative of either gross necropsy diagnosis or final cause of death for the major causes of death with the noted exception of lameness. Again this is unsurprising. Clinical categories were derived from clinical signs noted by the AAV and are arguably not suited to be used as a possible classification of causes of death – they are simply a record of signs displayed by animals and noted by the AAV. It is difficult to restrain individual animals for detailed examination. Clinical category information is based on intermittent visual observations of cattle while in pens. Clinical observations are likely to be relatively non-specific and many sick cattle may show relatively little outward and obvious signs of illness until they are very sick. The clinical categories with larger numbers of cases recorded were conditions such as recumbency and weakness, ill-thrift / shy feeder. These terms are very non-specific and this is reflected in the fact that final causes of death for animals with these clinical category records were distributed widely across several different causes of death and in fact included many cases of respiratory disease. Clinical categories such as lameness were much more specific, probably because lame animals tend present with visually obvious changes in appearance, gait and behaviour.

Table 11: Summary table showing cross classification of 215 mortality cases by clinical category (representing ante-mortem signs or syndromes) and gross necropsy diagnosis

	Clinical category as recorded on necropsy forms											
Gross necropsy diagnosis	**Respiratory signs**	**Lameness**	**Misadventure**	**Recumbent or weak**	**Ill-thrift / shy feeder**	**Neurologic signs**	**Pink eye**	**Sudden death**	**Urinary signs**	**No previous clinical signs**	**No clinical category recorded**	**Total**
Respiratory disease	12	3	1	13	13	1		1		54	2	**100**
Inflamm. of joints/muscle		17								3		**20**
Inappetence		1		5	7					2		**15**
Misadventure		2	6			1				4		**13**
Enteric disease	1			1	1	2				5		**10**
Anaesthetic complication		4										**4**
Metabolic disease		1				3						**4**
Cardiovascular disease	2											**2**
Urinary tract disease				1					1			**2**
Encephalitis						1						**1**
Neurogenic lameness		1										**1**
No significant lesions		1		11	1	2				7		**22**
Not recorded		1	1		1		1			4	13	**21**
Total	**15**	**31**	**8**	**31**	**23**	**10**	**1**	**1**	**1**	**79**	**15**	**215**

Table 12: Summary table showing cross classification of 215 mortality cases by clinical category (representing ante-mortem signs or syndromes) and final cause of death (after completion of all pathology examinations and tests).

	Clinical category											
Final cause of death	**Respiratory signs**	**Lameness**	**Misadventure**	**Recumbent / weakness**	**Ill-thrift / shy feeder**	**Neurological signs**	**Pink eye**	**Sudden death**	**Urinary signs**	**No previous clinical signs**	**No clinical category recorded**	**Total**
Respiratory disease	9	3	1	13	13	2		1		57	8	**107**
Musculoskeletal / injury		24	7	1						1		**33**
Ketosis		1		2	6				1	2		**12**
Septicaemia	1	2		2	1					3	2	**11**
Enteric disease	3			2		2				1	2	**10**
Heat Stroke	1									1		**2**
Other	1			1		1	1			2		**6**
No diagnosis		1		10	3	5				12	3	**34**
Total	**15**	**31**	**8**	**31**	**23**	**10**	**1**	**1**	**1**	**79**	**15**	**215**

Table 13: Summary table showing cross classification of 215 mortality cases by gross necropsy diagnosis and final cause of death (after completion of all pathology examinations and tests). NSL= no significant lesion, NR= not recorded.

	Gross necropsy diagnosis													
Final cause of death	**Resp. dis.**	**Inflam. of joints /muscle**	**Inappet.**	**Misadven.**	**Enteric dis.**	**Anaesth. Complic.**	**Metabolic dis.**	**Cardiovasc. dis.**	**Urinary tract dis.**	**Enceph.**	**Neuro. lameness**	**NSL**	**NR**	**Total**
Respiratory disease	90	1	2	3	1							2	8	**107**
Musculoskeletal / injury	1	15		7		4	1				1	2	2	**33**
Ketosis			10		1				1					**12**
Septicaemia	2	3	1									3	2	**11**
Enteric disease	1				5			1				1	2	**10**
Heat Stroke	2													**2**
Other				2				1	1			1	1	**6**
No diagnosis	4	1	2	1	3		3			1		13	6	**34**
Total	**100**	**20**	**15**	**13**	**10**	**4**	**4**	**2**	**2**	**1**	**1**	**22**	**21**	**215**

Resp. dis. = Respiratory disease; Inflam. = Inflammation; Inappet. = Inappetence; Misadven.= Misadventure; Enteric dis. = Enteric disease; Anaesth. Complic. = Anaesthetic complication; Metabolic dis. = Metabolic disease; Cardiovasc. dis. = Cardiovascular disease; Urinary tract dis. = Urinary tract disease; Enceph. = Encephalitis; Neuro. lameness = Neurogenic lameness; NSL = no significant lesion; NR = not recorded.

7.10 Diagnostic test assessment for causes of death

A diagnostic test is any procedure or process intended to detect a sign, substance, response or disease. In order to be a useful test, the process must be better than random chance in detecting the condition (i.e. perform better than flipping a coin to determine whether the disease is present).

An important outcome from this project will be to use the findings to improve the way AAVs (and stockpersons) manage animal health for exported livestock. On board an export vessel, AAVs are expected to use their observational skills to monitor animal health and also to use appropriate procedures (clinical observations and necropsies) to identify causes of death.

This project involved protocols for collection of various tissues and biological samples from necropsies conducted during voyages. Samples were returned to Australia and examined by veterinary pathologists using a combination of routine (histology) and advanced (qPCR) diagnostic procedures to determine the cause of death. Causes of death recorded by pathologists were based on all available data for each animal (clinical information, gross necropsy findings, photographs, histology, and qPCR results).

Statistical methods used to assess diagnostic test performance can be used to explore the on-board investigations that an AAV can routinely conduct to see how effective they might be in diagnosing the major causes of death in cattle on export voyages.

The data collected from AAVs provides two separate opportunities to measure diagnostic performance in detecting the major causes of death. The first is in the clinical category information which represents the knowledge about likely causes of death at the time that a dead animal is detected. Clinical category information is derived from observations about the animal from prior to death i.e. signs the animal may have shown prior to death. Clinical category information does not include any information derived from the gross necropsy.

The second opportunity to measure diagnostic performance for detecting the cause of death is to use the results from the gross necropsy as represented by the gross necropsy diagnosis information. This information is recorded on completion of the gross necropsy and is drawn from all available information the AAV has to hand at that time (clinical category information, if available, plus results of gross necropsy examination). It does not include any information from investigations conducted by pathologists in Australia.

Both clinical category information and gross necropsy diagnosis can be assessed as separate diagnostic tests aimed at detecting the cause of death. These two tests can each be compared to the final cause of death as determined by the veterinary pathology team. In the formal methodology of diagnostic test assessment, the final cause of death is assumed to be the gold standard test and the candidate diagnostic tests under assessment (clinical category and gross necropsy) are each compared to the gold standard test. Statistical procedures for assessing diagnostic test performance are well described. [17]

Data drawn from results presented in this chapter was used in routine calculations to produce outputs commonly used in assessment of diagnostic tests including sensitivity (Se), specificity (Sp), predictive values, apparent prevalence and true prevalence. Calculations

[17] Gardner and Greiner (1999); Dohoo, Martin, and Stryhn (2009)

were performed using the epidemiology toolbox app, available for free for various devices including computers and handheld devices. [18]

7.10.1 Respiratory disease detection using gross necropsy

Table 14 provides summary counts of classification of cases based on the gold standard test (columns) and the gross necropsy diagnosis (rows).

Table 14: Summary of classification of 215 cases of mortality by gold standard test (final cause of death) and the gross necropsy diagnosis outcome, using respiratory disease as the outcome of interest

Respiratory disease		Gold standard test Final cause of death		
		Disease +	Disease -	
Gross necropsy diagnosis	Test +	90	10	100
	Test -	17	98	115
		107	108	215

Test assessment assumes that the gold standard test is 100% accurate. The gold standard test has detected 107 animals with respiratory disease as the true cause of death and 108 animals where the cause of death was not respiratory disease (classified as disease negative when respiratory disease is the outcome of interest).

Gross necropsy diagnosis is then assessed based on the level of agreement between the gross necropsy diagnosis and the gold standard test.

A perfect diagnostic test would have 100% agreement with the gold standard test.

A test positive (+) result for gross necropsy diagnosis means a gross necropsy diagnosis of respiratory disease and a test negative (–) result means that respiratory disease was not recorded as the gross necropsy diagnosis (some other diagnosis was recorded).

As shown in Table 14, gross necropsy diagnosis failed to detect 17 animals that died from respiratory disease (classified as disease + on the gold standard test and as disease – on the gross necropsy diagnosis), and the gross necropsy diagnosis incorrectly assigned 10 animals to respiratory disease when the gold standard test classified these 10 animals as having died from a cause other than respiratory disease. Both tests correctly classified the remaining 98 animals as not having respiratory disease.

Gross necropsy diagnosis therefore correctly classified most of the respiratory disease cases and incorrectly classified some disease free animals as being diseased and some diseased animals as being disease free.

Table 15 provides estimates of the various measures that can be used to assess diagnostic test performance when assessing gross necropsy diagnosis as a method for detecting respiratory disease as a cause of death.

[18] Thanh Hoa Nguyen, 2012. Epidemiology toolbox, https://itunes.apple.com/us/app/epidemiology-toolbox/id477457802?mt=8

Table 15: Statistical measures of diagnostic performance for gross necropsy diagnosis as a test for detecting respiratory disease as a cause of death in cattle. Based on data in Table 14. Se= sensitivity; Sp=specificity; PPV = positive predictive value; NPV = negative predictive value; App Prev = apparent prevalence; True Prev = true prevalence; CI = Confidence Interval.

Parameter	Estimate	95% CI	
		Lower	Upper
Se	0.84	0.76	0.9
Sp	0.91	0.84	0.95
PPV	0.9	0.83	0.94
NPV	0.85	0.78	0.91
App Prev	0.47	0.4	0.53
True Prev	0.5	0.43	0.56

Sensitivity and specificity are measures of diagnostic test accuracy.

Sensitivity is the probability of a test positive result (Test +), given that the disease is present (Disease +). Sensitivity measures the ability of the test to correctly detect as test positive those animals that are known to have the disease of interest. Gross necropsy diagnosis has a sensitivity of 0.84, meaning that 84% of animals that truly died from respiratory disease would be correctly classified as test positive using gross necropsy.

Specificity is the probability of a test negative result (Test -), given that the disease is absent (Disease -). Specificity measures the ability of the test to correctly detect as test negative those animals that are known to be disease free. Gross necropsy diagnosis has a specificity of 0.91, meaning that 91% of animals that truly did not die from respiratory disease would be correctly classified as test negative using gross necropsy.

One minus the Se is a measure of false negatives, meaning those animals that truly died of respiratory disease and that were classified as negative on gross necropsy (n=17). These animals are called false negatives because the gross necropsy diagnosis has classified them as test negative when in fact the gold standard test has classified those 17 animals as dying from respiratory disease. Gross necropsy has a false negative rate of 0.16 or 16%.

One minus the Sp is a measure of false positives. These animals are called false positives because the gross necropsy diagnosis has classified them as test positive when in fact the gold standard test has classified those 10 animals as dying from causes other than respiratory disease (Disease -). Gross necropsy has a false positive rate of 0.09 or 9%.

While Se and Sp are useful measures of diagnostic test accuracy, they are interpreted only in the knowledge of the true disease status of each animal.

Predictive value measures provide slightly different information that is more direct use for an AAV performing a gross necropsy where the true cause of death is unknown. This is the situation on board a ship during an export voyage. Predictive values provide a measure of the probability of a disease outcome (true disease positive or negative) given the results of the diagnostic test (gross necropsy). Predictive value estimates are dependent in part on the prevalence of the condition of interest in the target population. This is an important concept to take note of particularly if it is possible that the same disease may be occurring in different

populations at different frequencies. For example, it may be possible that respiratory disease occurrence might be different in one specific group of animals compared to another. If the prevalence of a disease is different in different populations then applying the same diagnostic test to those different populations may produce different predictive values because predictive value estimates are influenced by the prevalence.

For respiratory disease, gross necropsy has a positive predictive value (PPV) of 0.9 and a negative predictive value (NPV) of 0.85.

This means that if a gross necropsy is performed during a voyage and the gross necropsy diagnosis is respiratory disease, then that animal has a 90% probability of truly having respiratory disease as the cause of death (positive predictive value).

Conversely if a gross necropsy is performed and the gross necropsy diagnosis is not respiratory disease, then that animal has an 85% probability of having died from a cause other than respiratory disease (negative predictive value).

Having information on diagnostic test performance and gold standard test outcomes also provides a more detailed understanding of prevalence estimates. The prevalence of respiratory disease as a cause of death is the probability that a mortality case died of respiratory disease.

The diagnostic test result (gross necropsy) classified animals as test positive or test negative and using these numbers we can generate a prevalence estimate of respiratory disease: 100/215 = 47%.

The term ***apparent prevalence*** is used to refer to the prevalence estimate derived from the diagnostic test (gross necropsy) because it is really a prevalence of a positive test result and not the prevalence of true disease since the diagnostic test is not perfect.

If a gold standard test result is available then these results provide an estimate of the ***true prevalence***, based on the final cause of death results.

7.10.2 Respiratory disease detection using clinical category information

Table 16: Summary of classification of 215 cases of mortality by gold standard test (final cause of death) and the clinical category information outcome, using respiratory disease as the outcome of interest.

Respiratory disease		**Gold standard test Final cause of death**		
		Disease +	**Disease -**	
Clinical category	**Test +**	9	6	15
	Test -	98	102	200
		107	108	215

Table 17: Statistical measures of diagnostic performance for clinical category information as a test for detecting respiratory disease as a cause of death in cattle. Based on data in Table 16. Se= sensitivity; Sp=specificity; PPV = positive predictive value; NPV = negative predictive value; App Prev = apparent prevalence; True Prev = true prevalence; CI = Confidence Interval.

Parameter	Estimate	95% CI	
		Lower	Upper
Se	0.08	0.04	0.15
Sp	0.94	0.88	0.97
PPV	0.6	0.36	0.8
NPV	0.51	0.44	0.58
App Prev	0.07	0.04	0.11
True Prev	0.5	0.43	0.56

The results clearly show that clinical category information alone is not useful for gaining any reasonable understanding of the extent of respiratory disease as a cause of death on export voyages. The sensitivity in particular is very low indicating that clinical category information is particularly poor at detecting those animals that truly have died of respiratory disease.

The predictive values appear to be little better than tossing a coin to determine the probability of a disease outcome given the findings of the clinical category information.

The apparent prevalence estimate (proportion of all deaths that are due to respiratory disease) when based on clinical category information is seriously inaccurate and an under-representation of the true prevalence.

The results confirm that clinical category information is insufficient to provide a reasonable understanding of the contribution of respiratory disease to mortality on export voyages and that gross necropsy diagnosis is essential to achieve a good understanding of the contribution of respiratory disease to voyage mortalities.

7.10.3 Musculoskeletal / injury detection using gross necropsy

Table 18: Summary of classification of 215 cases of mortality by gold standard test (final cause of death) and the gross necropsy diagnosis, using musculoskeletal / injury as the outcome of interest. Gross necropsy diagnoses of inflammation of joints, misadventure, anaesthetic complications and neurogenic lameness were combined to represent musculoskeletal / injury conditions.

Musculoskeletal / injury		Gold standard test Final cause of death		
		Disease +	Disease -	
Gross necropsy diagnosis	**Test +**	26	11	37
	Test -	7	171	178
		33	182	215

Table 19: Statistical measures of diagnostic performance for gross necropsy diagnosis as a test for detecting musculoskeletal / injury conditions as a cause of death in cattle. Based on data in Table 18. Se= sensitivity; Sp=specificity; PPV = positive predictive value; NPV = negative predictive value; App Prev = apparent prevalence; True Prev = true prevalence; CI = Confidence Interval.

Parameter	Estimate	95% CI	
		Lower	Upper
Se	0.79	0.62	0.89
Sp	0.94	0.9	0.97
PPV	0.7	0.54	0.83
NPV	0.96	0.92	0.98
App Prev	0.17	0.13	0.23
True Prev	0.15	0.11	0.21

A gross necropsy diagnosis of musculoskeletal / injury conditions has a very high Sp and a very high NPV. This indicates that when the gross necropsy diagnosis does not record lameness, there is a very high level of confidence that the animal did not die of musculoskeletal / injury conditions (96% based on NPV).

The Se is only moderate (0.68) indicating that 79% of animals that were classified as having musculoskeletal / injury conditions as the final cause of death (true disease positive) were classified as having musculoskeletal / injury conditions as the gross necropsy diagnosis.

7.10.4 Musculoskeletal / injury condition detection using clinical category information

Table 20: Summary of classification of 215 cases of mortality by gold standard test (final cause of death) and the clinical category information outcome, using musculoskeletal / injury conditions as the outcome of interest.

Musculoskeletal / injury		Gold standard test Final cause of death		
		Disease +	Disease -	
Clinical category	Test +	31	8	39
	Test -	2	174	176
		33	182	215

Table 21: Statistical measures of diagnostic performance for clinical category information as a test for detecting lameness as a cause of death in cattle. Based on data in Table 22. Se= sensitivity; Sp=specificity; PPV = positive predictive value; NPV = negative predictive value; App Prev = apparent prevalence; True Prev = true prevalence; CI = Confidence Interval.

Parameter	Estimate	95% CI	
		Lower	Upper
Se	0.94	0.8	0.98
Sp	0.96	0.92	0.98
PPV	0.79	0.64	0.89
NPV	0.99	0.96	1
App Prev	0.18	0.14	0.24
True Prev	0.15	0.11	0.21

There is a striking difference between these results for clinical category detection of musculoskeletal / injury conditions as a cause of death and the results for clinical category detection of respiratory disease as a cause of death.

Clinical detection of musculoskeletal / injury conditions performs well as gross necropsy diagnosis as a test for detection of musculoskeletal / injury conditions as a cause of death.

The findings support the suggestion that musculoskeletal / injury conditions is a very different type of disease to respiratory disease, mainly because musculoskeletal / injury conditions cases are more likely to have been observed and recorded in clinical category records. Musculoskeletal and injury conditions are more obvious and noticeable both ante-mortem and post-mortem.

It is interesting to note that musculoskeletal / injury conditions has a moderate PPV. The PPV of 0.79 means that once a clinical diagnosis of musculoskeletal / injury conditions is made as a cause of death, there is a 79% probability that musculoskeletal / injury conditions truly was the cause of death.

This does not mean that the musculoskeletal / injury conditions observation was not accurate. It is likely to reflect the fact that animals that are lame or injured become increasingly susceptible to other serious diseases that may ultimately cause death (or necessitate euthanasia). The role of musculoskeletal / injury conditions as a cause of death has some relatively complex dimensions.

If the ultimate disease or condition that is classified as the cause of death would not have occurred had the animal not been lame or injured, then undoubtedly the lameness/injury played an important role in determining the animal's subsequent mortality, even if it was not literally the thing that ultimately killed the animal.

Extending the counter-factual argument a little further poses the question that if an initial clinical classification of musculoskeletal / injury conditions had been either prevented or successfully treated, would this have prevented the occurrence of other diseases likely to occur in lame/injured animals and that may result in death. The point of this is that prevention and or treatment of lameness/injury may reduce mortalities attributed to causes of death other than lameness. This is not novel and is supported by anecdotal information

from experienced AAVs and exporters. It is pleasing to see the results of statistical analyses supporting well accepted hypotheses and anecdotal opinions since it adds confidence in the interpretation of the findings and in using these results to inform preventive strategies.

It is also interesting that the apparent prevalence for musculoskeletal / injury conditions is much closer to the true prevalence estimate than was observed for respiratory disease. The apparent prevalence is a little higher than the true prevalence, reflecting the same issue as discussed in the previous paragraph.

When the diagnostic information for musculoskeletal / injury conditions is considered, it suggests that musculoskeletal / injury conditions can be effectively detected as a cause of death without requiring necropsy examination for diagnostic classification – in contrast to respiratory disease.

This is not to say that lame animals that die should not be subjected to necropsy. The point is that necropsy of lame/injured animals that die is of value not only to confirm a necropsy diagnosis of lameness/injury as a cause of death, but to investigate other causes of death such as respiratory disease that can only be diagnosed effectively at necropsy and not by clinical category information alone. This information can be used potentially to fine tune the approach to necropsy to make it more efficient without reducing its diagnostic effectiveness. This will be revisited in more detail in later sections of this report.

7.10.5 Inappetence detection using gross necropsy

Inappetence at gross necropsy was mapped most closely to ketosis as a final cause of death.

Table 22: Summary of classification of 215 cases of mortality by gold standard test (final cause of death) and the gross necropsy diagnosis, using inappetence/ketosis as the outcome of interest.

Ketosis vs inappetence		**Gold standard test Final cause of death**		
		Disease +	**Disease -**	
Gross necropsy diagnosis	**Test +**	10	5	15
	Test -	2	198	200
		12	203	215

Table 23: Statistical measures of diagnostic performance for gross necropsy diagnosis as a test for detecting inappetence/ketosis as a cause of death in cattle. Based on data in Table 22. Se= sensitivity; Sp=specificity; PPV = positive predictive value; NPV = negative predictive value; App Prev = apparent prevalence; True Prev = true prevalence; CI = Confidence Interval.

Parameter	Estimate	95% CI	
		Lower	Upper
Se	0.83	0.55	0.95
Sp	0.98	0.94	0.99
PPV	0.67	0.42	0.85
NPV	0.99	0.96	1
App Prev	0.07	0.04	0.11
True Prev	0.06	0.03	0.1

The Se and Sp measures are moderate to high.

The PPV is only moderate and reflects in part the fact that the prevalence of the condition is generally low (6-7%) as well as the fact that some animals classified with inappetence at gross necropsy were ultimately determined to have died from some other cause as the final cause of death. It is recognised that inappetence / ketosis can be an important cause of mortality risk under certain conditions such as shipments of pregnant cattle or older cows.

As an example of the impact of prevalence on estimates of PPV, the PPV was re-calculated using a standard formula involving Se, Sp and prevalence [19], while holding the Se and Sp constant at the values shown in Table 23 and allowing prevalence to vary from 0.1 to 0.4.

$$PPV = \frac{P*Se}{(P*Se)+(1-P)*(1-Sp)}$$ P=prevalence

As prevalence rises from 0.1, 0.2, 0.3, 0.4 the PPV rises to 0.82, 0.91, 0.95, and 0.97, respectively.

7.10.6 Enteric disease detection using gross necropsy

Table 24: Summary of classification of 215 cases of mortality by gold standard test (final cause of death) and the gross necropsy diagnosis, using enteric disease as the outcome of interest.

Enteric disease		Gold standard test Final cause of death		
		Disease +	Disease -	
Gross necropsy diagnosis	**Test +**	5	5	10
	Test -	5	200	205
		10	205	215

[19] Gardner and Greiner (1999)

Table 25: Statistical measures of diagnostic performance for gross necropsy diagnosis as a test for detecting enteric disease as a cause of death in cattle. Based on data in Table 24. Se= sensitivity; Sp=specificity; PPV = positive predictive value; NPV = negative predictive value; App Prev = apparent prevalence; True Prev = true prevalence; CI = Confidence Interval.

Parameter	Estimate	95% CI	
		Lower	Upper
Se	0.5	0.24	0.7
Sp	0.98	0.94	0.99
PPV	0.5	0.24	0.76
NPV	0.98	0.94	0.99
App Prev	0.05	0.03	0.08
True Prev	0.05	0.03	0.08

While the NPV is very high, the PPV for a gross necropsy diagnosis of enteric disease was relatively poor. This is likely to be the result of a relatively poor Se as well as the low prevalence of the condition. Enteric conditions include diseases such as salmonellosis and under favourable conditions (shedding animals, rough voyage with associated stress and presence of concurrent disease) it is possible that multiple cases of enteric disease could occur.

If Se and Sp are held at the values above and prevalence is increased to 0.1 and 0.2, the PPV does rise to 0.74 and 0.86, respectively.

7.10.7 Other diagnostic classifications

Clinical categories of *recumbency and/or weakness* and *ill thrift and/or shy feeder* accounted for 31 and 23 animals, respectively (Section 7.5 and Table 11) but there was very little relationship between animals in either of these two clinical categories and either gross necropsy diagnosis (Table 11) or final cause of death (Table 12). The information suggests that these two clinical categories are non-specific, descriptive terms that are not associated with particular diseases.

There were a range of other conditions that were listed either as clinical categories or as gross necropsy diagnoses, however the number of animals recorded against each of these other conditions was so small as to preclude effective assessment of the diagnostic performance of these conditions (association between clinical category or gross necropsy diagnosis and the gold standard of final cause of death).

7.11 Discussion

The project design called for enrolling 40 voyages and completing necropsies at three broad levels: 75 necropsies with detailed examination of all collected tissues, 75 necropsies with some tissues collected and with limited pathology testing and up to 100 necropsies without any collection of samples (gross necropsy only). These figures assumed a total of 250 necropsies but of these only about 150 were expected to result in samples being imported for pathology testing.

Very early in Stage 3 the project team saw that voyage enrolment rates were likely to result in fewer than 40 voyages and as a result an effort was made to encourage necropsy and

collection of biological samples from as many dead animals as possible. The project ended with necropsy forms being submitted for 215 deaths and necropsies and collection of samples were achieved on 197 of these. All samples were processed and examined for histology and molecular testing where ever possible.

The final result was that we collected pathology data and information from more animals than we had planned to while enrolling fewer voyages than we had planned to.

Since the purpose of the necropsies was to establish and describe common causes of death, the project team felt that the project had collected sufficient samples to be confident that we had achieved this goal.

Respiratory disease was the most common cause of mortality identified in the current study, accounting for about 50% of the 215 deaths for which information was obtained and 59.1% of the deaths for which a final diagnosis could be made.

Estimates of the proportion of deaths attributed to respiratory disease in feedlot studies in Canada range from 46-55% [20] and in the United States from 55–75%. [21] A recent survey of Australian cattle feedlots reported that respiratory disease and musculoskeletal conditions were the two most common causes of death, accounting for 53% and 15% of all mortalities, respectively. [22] These findings are consistent with the results from the current project.

Histological evidence of concurrent respiratory disease was also present in an average of 33% of animals for which respiratory disease was not considered the primary cause of death.

There were two important differences between our findings and those of an earlier study of causes of death in four cattle export voyages to the Middle East. [23] The most important cause of death in cattle reported in the Norris et al study was heat stroke, followed by trauma (or musculoskeletal disease and injury) and respiratory disease. The two key differences were that we found respiratory disease accounted for a higher proportion of mortalities and our findings suggested that heat stroke was a very uncommon cause of death in the population of export cattle we followed.

It is not clear why respiratory disease accounted for a lower proportion of mortalities in the Norris et al study in comparison to the current project. There were differences in design with the earlier study involving designated research personnel accompanying each selected voyage, conducting necropsies and generally assuming responsibility for all sample and data collection. The Norris et al study was conducted about a decade prior to this study and there have been changes in management of shipboard conditions and animal selection since that time including implementation of heat stress management associated with *HotStuff*. Our project involved regular AAVs doing the data collection and performing necropsies on board ship. Our project involved pathologists examining all tissues collected during voyages and the results from these examinations indicated a high level of agreement with the AAV classifications of causes of death based on gross necropsy alone. In addition, our project included one voyage where a research team member accompanied the voyage and

[20] Edwards (1996); Gagea et al. (2006)
[21] Loneragan et al. (2001)
[22] Perkins (2013)
[23] Norris et al. (2003)

conducted necropsies and the results from this voyage were consistent with results from all other voyages. As a result we are generally confident in the findings from this project. This suggests that the differences between the earlier paper [24], and the current project were due to changes in the interplay of various causal factors (animal, ship, climate, pathogen, management etc) that may influence mortality risk.

Quantitative PCR assay was used in the present study to detect potential respiratory pathogens in samples collected from the nose and lungs of cattle on board export vessels. The prevalence of viruses of interest (BCoV, BoHV-1, BRSV, BVDV, BPIV-3) was less than 15% in both nasal and lung samples. The prevalence of these viruses was higher in animals with clinical or histological evidence of respiratory disease than those without, with the exception of BRSV in lung samples.

In the pathogenesis of BRD, viruses play a primary role, causing damage to the respiratory tract and facilitating secondary invasion by bacteria that may go on to cause a fatal bacterial pneumonia. It is possible that by the time that an animal develops severe disease and dies from a secondary bacterial pneumonia, that much of the evidence to document an initial or primary viral infection and the ability to detect specific primary viruses may be obscured.

As a result our findings are considered consistent with the general understanding of respiratory disease in cattle where primary viral infection is often followed by secondary bacterial infection.

We have reported a molecular diagnosis of BCoV in association with respiratory disease in Western Australian and South Australian cattle that were sampled during this study.

In a separate publication, BCoV was recently reported in association with respiratory disease in cattle on a property on the south coast of New South Wales, Australia. [25] BCoV appears to be emerging as a potential cause of respiratory disease in cattle and more work is required to determine whether there are strain differences between enteric and respiratory syndromes associated with BCoV infection and to assess relative importance of BCoV compared to other respiratory viruses and also potential control and prevention strategies.

We reported a tendency for a statistical association between BVDV and respiratory disease. A number of animals that tested positive for BVDV came from consignments in which acutely or persistently infected animals had been previously identified and removed from the consignment prior to loading, using the pestivirus antigen capture ELISA or PACE test. Our findings suggest that current pre-export testing strategies for BVDV will not eliminate the risk of BVDV circulation amongst cattle being assembled for export.

We found significant associations between a diagnosis of BRD as a cause of death and detection of each of the four bacterial pathogens of interest in lung tissue. Animals with histologic pneumonia were more likely to have a positive qPCR result for each of the four bacteria than animals without pneumonia. The strongest statistical association was reported for *Mycoplasma bovis* but associations for all four bacteria were statistically significant.

In addition, animals with histologic pneumonia were more likely to have three of the four bacteria detected in nasal swabs compared to animals without pneumonia (*M. bovis, M.*

[24] Ibid.
[25] Hick et al. (2012)

haemolytica, and *P. multocida*). All of these bacteria are known to be important causes of BRD in cattle.

We did not find an association between *M. bovis* detection and musculoskeletal conditions though *M. bovis* is well described as a cause of arthritis and tenosynovitis in cattle in addition to BRD. Relatively few samples from musculoskeletal lesions were collected during our study in comparison to the collection of respiratory disease samples and the lack of association in our study may have been due to sampling constraints.

We also reported a small number of cases with histologic changes consistent with myocarditis, pneumonia or thrombotic meningoencephalitis associated with *H. somni* infection. Myocarditis, BRD, polyarthritis and thrombotic meningoencephalomyelitis are described as common clinical manifestations of *H. somni* infection in feedlot cattle. [26]

We reported heat stroke as the primary cause of death in only two of 215 animals (less than 1%) though hyperthermia secondary to other disease conditions (pneumonia and septicaemia) was described in a further 9 animals. The reduced occurrence of heat stroke as a cause of death in comparison to earlier studies [27], may be due to factors such as misclassification, changes in selection and management of cattle both prior to and during the voyage, and changes in airflow and climatic management on-board export vessels.

Musculoskeletal conditions including lameness and injury, was the second most common cause of death in the current project. The proportion of deaths attributed to musculoskeletal conditions and injury in our study (15.3%) was lower than previous estimates of 23% [28], and 25%. [29] This is likely due to a combination of factors including things such as animal selection, change in type of cattle being exported and improved conditions and management during the voyage.

Lameness may occur at any stage during the export supply chain, often occurring as a result of a combination of injury and abrasive or rough flooring. During the voyage, injuries are more common during rough weather particularly in combination with heavier animals and reduced stocking density (more opportunity for animal movement or falls). [30] Difficulty in separating and treating individual animals during the voyage and ongoing movement of the vessel and local conditions (wet bedding, abrasive flooring) mean that musculoskeletal injuries that may heal relatively well in a land-based environment may develop into serious and potentially life-threatening conditions on-board an export ship. A number of cattle with musculoskeletal conditions were euthanased because they were not able to recover in time to be unloaded at a destination port, reflecting the difficulty in managing these conditions on board a ship. Recommendations for best practice for cattle selection and bedding management to reduce the incidence of lameness have been made in previous industry reports [31], and in Commonwealth mortality investigation reports as described in Shiell et al. [32]

[26] Van Donkersgoed et al. (1994)
[27] Norris et al. (2003)
[28] Hedlefs (1988)
[29] Norris et al. (2003)
[30] Banney, Henderson, and Caston (2009); Hedlefs (1988)
[31] Banney, Henderson, and Caston (2009)
[32] Shiell, Perkins, and Hewitt (2014)

This project involved AAVs performing routine clinical work during the voyage including conducting necropsies and collecting samples. Samples were imported back into Australia and examined by veterinary pathologists to establish cause of death. An important outcome for this project was to use the findings to assess and improve the ability of AAVs to accurately classify causes of death without having to import biological samples back into Australia and have them examined by a pathologist.

Our findings confirm that AAVs are very capable of accurately assigning deaths to the appropriate primary cause using gross necropsy alone.

Our findings suggest that ante-mortem clinical observations on animals are not an effective way to classify or identify the cause of death for animals that subsequently die, with the possible exception of musculoskeletal and injury conditions. Between 40-50% of deaths in our study had no ante-mortem clinical disease information recorded suggesting that many deaths occur without any prior information on likely diseases. For those animals that died and that had any ante-mortem information recorded, ante-mortem clinical records were not useful in identifying the cause of death except for musculoskeletal conditions such as trauma and lameness.

Lameness and musculoskeletal injury severe enough to result in euthanasia or death, was most effectively detected using ante-mortem clinical information, followed by gross necropsy. Our findings also suggest that a number of animals with severe lameness conditions may develop secondary diseases perhaps as a result of being compromised by lameness.

In contrast, respiratory disease is only poorly detected by ante-mortem clinical information, however it is well detected using gross necropsy.

Other important causes of death (inappetence, enteric disease) had very high negative predictive values with gross necropsy but lower positive predictive values, indicating that gross necropsy can confidently be used to rule these conditions out and less confidently to diagnose them as the primary cause of death.

The important practical application of this information is that AAVs can confidently classify deaths according to the most important likely causes of death in export cattle and that this will require a combination of ante-mortem records focussing particularly on lameness conditions and gross necropsy for other conditions, most particularly respiratory disease.

8 Results - Stage 4 – Nasal shedding and seroprevalence of potential pathogens

Stage 4 objectives were designed to incorporate sampling of animals during the assembly feedlot stage of the export supply chain in order to understand possible levels of exposure to potential respiratory disease pathogens as animals are sourced and assembled, and where possible to relate these findings to subsequent disease occurrence during export.

The general purpose was to investigate the contribution of pre-existing or concurrent disease, management practices, seasonality or other factors to development of clinical disease once animals were on-board. In addition we wanted to describe processes for handling and health management at assembly depots.

8.1 Description of management of cattle in assembly depots

8.1.1 Sourcing

Most exporters source cattle through in-house buyers, although buyers from agency firms (e.g. Landmark, Elders) may also be used particularly if the exporter is sourcing cattle outside their usual geographical area.

Sourcing of cattle may begin one week to months before the ship sails, depending on the type of cattle being exported and the importing country requirements. For example, cattle may be sourced 7+ days prior for South East Asian markets, and up to 45 days prior for Middle Eastern and European markets.

The majority of cattle are bought directly off farm to comply with the property of origin residency requirements stipulated in the importing country import protocol. Shipments typically comprise animals from multiple properties with each property supplying up to five percent of the animals on the shipment.

Cattle may be purchased through saleyards but only if this does not contravene property of origin residency requirements.

8.1.2 Protocolling

Animals sourced for export must meet with various requirements as stipulated in the Australian Standards for the Export of Livestock (ASEL) and the importing country's protocol.

Protocolling refers to the various checks and testing procedures that may be undertaken to ensure that animals do comply with these requirements. Usually, protocolling is done while animals are being held in an AQIS registered premise (assembly depot), although some procedures, such as pregnancy testing, may be done at the property of origin.

Where necessary during protocolling animals will be individually restrained in a race or crush for examination and sampling for procedures including:

- scanning of individual electronic identification tags (RFID/NLIS) and application of visual eartags;
- taking a blood sample for serological testing;
- measurement of liveweight;
- administration of required treatments such as vaccination, drench or prophylactic antibiotics; and
- treatment of minor conditions detected during handling such as ophthalmic conditions.

8.1.3 Induction into assembly depot

Cattle must be assembled in an AQIS registered premise for a defined period of time. This is to allow the animals to recover from the stress of transport and become accustomed to shipboard feed rations.

Due to the variability and seasonality of the live export trade many registered premises have dual purposes. They operate as pre-export assembly depots when required, and fattening feedlots for non-export animals at other times. All livestock entering a registered premise may only leave that facility for export or domestic slaughter.

During their time in the assembly depot all of the protocolling procedures will be finalised to ensure that animals meet the preparation and health requirements set out in the importing country import protocol.

After protocolling animals are drafted into groups ('lines') according to one or more of the following:

- type (*Bos taurus* vs *Bos indicus*);
- sex;
- purpose (feeder, slaughter, breeder);
- weight; and
- breed.

Some lines of animals will receive additional treatments (for example prophylactic antibiotics), in the lead up to loading either as part of the protocol or at the exporter's discretion. These are usually animals that are considered to be at a higher risk of developing disease during the voyage, particularly respiratory disease.

8.1.4 Assembly period

During the assembly period cattle are kept in large pens typical of a feedlot. Stocking densities must meet minimum ASEL requirements for square-metres per head.

Pens have concrete or compacted dirt floors, water troughs and feed bunkers, and may be covered or uncovered. The minimum quantity of feed that must be available is set out in ASEL. The type of ration is not specified in ASEL and, although it may be similar to that fed during the voyage, it is rarely the same. Hay may be mixed in with the ration to stimulate gut activity and encourage shy feeders to eat. Animals are usually fed twice a day.

Cattle are usually checked three times a day by the depot stockpersons. Sick animals may be removed to a hospital pen or treated in the pen. Dead livestock must be collected and disposed of on a daily basis as stipulated in ASEL. Dead animals are rarely necropsied to determine the cause of death.

Cattle may be held in the registered premises for 24 hours to 30 days depending on minimum ASEL requirements and pre-export quarantine requirements stipulated in the importing country protocol. Quarantine periods are generally shortest for slaughter cattle, longer for feeder cattle, and longest for breeder cattle. For short haul voyages (< 10 days) the ASEL minimum requirements are 24 hours for voyages with a single port of loading and discharge and one clear day (not including the day of arrival or day of departure) for voyages with multiple port loadings or discharges. For long-haul voyages (> 10 days) cattle must be held in the registered premises for no less than two clear days.

8.1.5 Rejects and Carry-overs

Animals which do not meet requirements may be rejected or carried-over.

Reject stock are those that do not meet requirements for the current shipment and are unlikely to (or will not) meet requirements for future consignments. Rejects are isolated from other consignment livestock, treated or euthanased if necessary, and removed from the depot, either by carcass disposal or domestic slaughter, in a timely manner.

Carry-over stock are those that do not meet requirements for the current consignment but may do so for a future consignment. These animals are held separate to the consignment livestock and, if treatment is successful, may be considered for inclusion in a future consignment.

8.1.6 Load-out

Cattle are typically held off water for up to 12 hours prior to loading and off food for 6-8 hours.

Generally animals are not individually weighed at load-out.

During load-out cattle are loaded onto trucks by line and also according to their final location on the ship. The road journey from the registered premises to port of loading must be less than 8 hours and most registered premises have typically road transit times to the port that are considerably shorter than the ASEL requirement.

Permission to leave for loading and the exit from the registered premises is only permitted when both the animals to be exported and export vessel have passed the necessary AQIS and Australian Maritime Safety Authority (AMSA) inspections. If in an extraordinary circumstance, loading cannot take place after cattle have already commenced their journey to the vessel, they can either return to the same facility, or if closer or offering a better welfare outcome, can be taken to another registered premises en-route.

8.2 Study population

The approach was to work with exporters to identify opportunities to collect samples from cattle during the assembly period and then once those cattle were loaded onto the ship to enrol that voyage in Stage 3 of the project.

Where possible the sampling strategy was designed to collect nasal swabs from a larger number of animals and serum samples from a subset of these cattle. Nasal swabs were tested using qPCR to detect the presence of genetic material from the major viral and bacterial respiratory disease pathogens of interest using the same tests as have been described in the previous chapter. Serum samples were analysed.

Samples were collected from cattle being prepared for four voyages (Table 26 and Table 27). Three of these voyages were enrolled in Stage 3 (voyage codes 5, 8 and 17) and therefore findings from the assembly testing were able to be related to causes of death information derived from the voyage and reported in the previous section. One voyage (voyage code 18) was not able to be enrolled in Stage 3 and therefore assembly sampling was not able to be related to information on causes of death from the voyage. Data were obtained on voyage details from the SMDB database for voyage 18 to allow a range of summary information to be reported for this voyage.

Nasal swab samples were collected from 1,484 animals, representing 1-5% of the total cattle loaded onto these voyages.

Cattle were sampled from eight separate assembly depots across three states (Table 27). Animals were believed not to have been vaccinated against respiratory disease prior to arriving at the depot.

Cohort sampling within one assembly depot involved systematic sampling of animals from the same property of origin based on PIC numbers in order to obtain sufficient numbers of animals from each of multiple cohorts, so that prevalence estimates may be produced for cohort and depot and voyage.

Table 26: Summary information for those voyages where cattle were sampled during the assembly period

Voyage code	Year	Season	Load port(s)	Discharge country	Voyage duration (days)	Cattle loaded (N)	Voyage deaths (n)	Assembly samples Swabs (n)	Assembly samples Serum (n)
5	2010	Summer	Fremantle	Turkey	23.6	19,990	148	410	0
8	2011	Autumn	Portland, Fremantle	Saudi Arabia, Turkey	34.9	17,449	52	413	0
17	2012	Autumn	Adelaide, Fremantle	Turkey	33.6	7,811	39	561	334
18	2012	Autumn	Portland, Fremantle	Russia	36.8	9,068	33	100	0
						54,318	272	1,484	334

Table 27: Details of assembly samples collected by cohort and voyage

Voyage code	Cohort code	Depot		Days before loading	Class	Assembly samples		
		State	Code			Swabs	Serum	Note
				(d)		(n)	(n)	
5	1	WA	4	NA	Feeder	10		a
	2	WA	4	9	Feeder	200		
	3	WA	1	5	Slaughter	200		
Total number of samples collected for voyage 5						***410***	***0***	
8	4	VIC	7	28	Feeder	102		
	5	WA	2	27	Feeder	112		
	6	WA	4	21	Slaughter	99		
	7	WA	3	19	Slaughter	100		
Total number of samples collected for voyage 8						***413***	***0***	
17	8a	WA	5	16	Feeder	25	25	b
	8b	WA	5	9	Feeder	18		c
	9	SA	8	9	Feeder	92	91	
	10	SA	8	8	Slaughter	20		
	11	WA	6	13	Feeder	75	75	
	12a	WA	5	12	Feeder	143	143	d
	12b	WA	5	3	Feeder	108		e
	13	WA	5	5	Slaughter	80		
Total number of samples collected for voyage 17						***561***	***334***	
18	14	WA	1	28	Breeder	100		
Total number of swabs collected for voyage 18						***100***	***0***	
Total number of samples collected overall						***1,484***	***334***	

Note:
a: ten cattle that were not loaded onto the voyage because of health concerns
b: cattle carried over in the assembly depot from the previous voyage, first of two samples from the same cohort.
c: second sampling from the same cohort, 7 days after the first sampling.
d: first sampling of newly arrived cattle.
e: second sampling of same cohort, 9 days after first sampling.

Voyages 5, 8 and 17 were made up of a mixture of feeder and slaughter cattle. Voyage 18 was a shipment of breeder cattle to the Russian Federation. Overall the class breakdown was 60% feeder, 34% slaughter and 6% breeder. Eighty-four percent of cattle were *Bos taurus* or *B. taurus* crosses and 16% were *Bos indicus* genotype. Eighty-seven percent of cattle were male (65% steer, 23% bull; 62% feeder, 38% slaughter) and 13% were female (57% breeder, 43% feeder).

Within voyage 17, nested sampling at two different time periods on the same cohorts was used to investigate whether the prevalence of viral and bacterial shedding changed with time (Table 27). Cohort 8 was a group of cattle that had entered the assembly depot in

preparation for the previous voyage and that were retained in the assembly depot and loaded onto the next available voyage (voyage 17). These animals are commonly described as ‘carried over’ or ‘held over’. A random and representative sample of animals from cohort 8 were sampled on two occasions with the second sampling occurring seven days after the first sampling.

Cohort 12 was a newly arrived group of cattle that were sampled initially on the day of arrival at the assembly depot and then sampled a second time nine days later.

It was not possible to select the same individuals for sampling at the first and second sampling sessions, but animals that were sampled at the second session were selected from pens containing animals that had been sampled at the first session for each of the two re-sampled cohorts.

Serum samples were collected from animals from 1 voyage (voyage 17). Serum and nasal swab samples collected from the same animal in another nested study aimed at comparing nasal swab test results to serum antibody test results from the same animals. Samples were collected at the time animals arrived at the assembly depot for four separate cohorts of animals from two states (Table 27).

8.3 Detection of respiratory pathogens

One or more of the viruses or bacteria of interest were detected in the nasal swabs from 1,150/1,484 (77%) of cattle. The remaining 334/1,484 (23%) animals were negative for all organisms tested.

Pathogen prevalence is reported as a percentage of animals tested within each sampled cohort and voyage (Table 28 and Table 29). There was significant variation between cohorts and voyages for prevalence of individual viruses and bacteria ($p<0.05$).

Bovine coronavirus (BCoV) was the most commonly detected virus in nasal swabs from cattle in the assembly feedlot, found in cattle from all voyages and in all but 2 of the cohorts tested (Table 28). Cohort-level prevalence for BCoV varied from 0% to 94% and the overall prevalence when all samples were combined was 40%.

Other respiratory viruses were detected at much lower prevalence levels and there were a number of cohorts that had no detections for one or more of the other four viruses (Table 28). There were only three occasions where a cohort level prevalence exceeded 10% for the other four viruses: twice for BVDV and once for BRSV.

Table 28: Count of number of nasal swabs by cohort and voyage, number of samples detected positive for each of five respiratory viruses, prevalence as a percentage and the 95% confidence interval (CI) for the prevalence.

Cohort & Voyage	Total	BCoV			BoHV-1			BRSV			BVDV			BPIV-3		
	N	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI
1	10	7	70.0	34.8-93.3	0	0.0	0.0-30.8	0	0.0	0.0-30.8	0	0.0	0.0-30.8	0	0.0	0.0-30.8
2	200	188	94.0	89.8-96.9	0	0.0	0.0-1.8	2	1.0	0.1-3.6	9	4.5	2.1-8.4	2	1	0.1-3.6
3	200	117	58.5	51.3-65.4	0	0.0	0.0-1.8	0	0.0	0.0-1.8	12	6.0	3.1-10.2	1	0.5	0.0-2.8
Voy 5	*410*	*312*	*76.1*	*71.7-80.1*	*0*	*0.0*	*0.0-1.0*	*2*	*0.5*	*0.06-1.8*	*21*	*5.1*	*3.2-7.7*	*3*	*0.7*	*0.2-2.1*
4	102	58	56.9	46.7-66.6	0	0.0	0.0-3.6	0	0.0	0.0-3.6	1	1.0	0.0-5.3	0	0.0	0.0-3.6
5	112	2	1.8	0.2-6.3	0	0.0	0.0-3.2	0	0.0	0.0-3.2	0	0.0	0.0-3.2	0	0.0	0.0-3.2
6	99	56	56.6	46.2-66.5	0	0.0	0.0-3.7	0	0.0	0.0-3.7	1	1.0	0.0-5.4	1	1	0.0-5.5
7	100	6	6.0	2.2-12.6	4	4.0	1.1-9.9	1	1.0	0.03-5.4	0	0.0	0.0-3.6	1	1	0.0-5.4
Voy 8	*413*	*122*	*29.5*	*24.2-34.2*	*4*	*1.0*	*0.3-2.5*	*1*	*0.2*	*0.0-1.3*	*2*	*0.5*	*0.1-1.7*	*2*	*0.5*	*0.1-1.7*
8a	25	2	8.0	1.0-26.0	2	8.0	1.0-2.6	0	0.0	0.0-13.7	3	12.0	2.5-31.2	0	0	0.0-13.7
8b	18	0	0.0	0.0-18.5	1	5.6	0.1-27.3	0	0.0	0.0-18.5	1	5.6	0.1-27.3	0	0.0	0.0-18.5
9	92	41	44.6	34.2-55.3	5	5.4	1.8-12.2	0	0.0	0.0-3.9	4	4.3	1.2-10.8	4	4.3	1.2-10.8
10	20	0	0.0	0.0-16.8	0	0.0	0.0-16.8	0	0.0	0.0-16.8	2	10.0	1.2-31.7	0	0.0	0.0-16.8
11	75	7	9.3	3.8-18.3	2	2.7	0.3-9.3	0	0.0	0.0-4.8	2	2.7	3.2-9.3	2	2.7	0.3-9.3
12a	143	32	22.4	15.8-30.1	0	0.0	0.0-2.5	0	0.0	0.0-2.5	7	4.9	2.0-9.8	4	2.8	0.8-7.0
12b	108	28	25.9	18.0-35.2	1	0.9	0.02-5.1	4	3.7	1.0-9.2	1	0.9	0.0-5.1	2	1.9	0.2-6.5
13	80	4	5.0	1.4-12.3	0	0.0	0.0-4.5	0	0.0	0.0-4.5	1	1.3	0.0-6.8	4	5	1.4-12.3
Voy 17	*561*	*114*	*20.3*	*17.1-23.9*	*11*	*2.0*	*1.0-3.5*	*4*	*0.7*	*0.2-1.8*	*21*	*3.7*	*2.3-5.7*	*16*	*2.9*	*1.6-4.6*
14	100	47	47.0	36.9-57.2	0	0.0	0.0-3.6	10	10.0	4.9-17.6	0	0.0	0.0-3.6	0	0.0	0.0-3.6
Total	*1,484*	*595*	*40.1*	*37.6-42.6*	*15*	*1.0*	*0.6-1.7*	*17*	*1.1*	*0.7-1.8*	*44*	*3.0*	*2.2-4.0*	*21*	*1.4*	*0.9-2.2*

Table 29: Count of number of nasal swabs by cohort and voyage, number of samples detected positive for each of four bacterial respiratory pathogens, prevalence as a percentage and the 95% confidence interval (CI) for the prevalence.

Cohort & Voyage	N	*Histophilus somni*			*Mycoplasma bovis*			*Mannheimia haemolytica*			*Pasteurella multiocida*		
		n	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
1	10	1	1.0	0.3-44.5	0	0.0	0.0-30.8	0	0.0	0.0-30.8	6	60.0	26.2-87.8
2	200	14	7.0	3.9-11.5	0	0.0	0.0-1.8	88	44.0	37.0-51.1	121	60.5	53.4-67.3
3	200	53	26.5	20.5-33.2	0	0.0	0.0-1.8	9	4.5	2.1-8.4	44	22.0	16.5-28.3
Voy 5	*410*	*68*	*16.6*	*13.1-20.5*	*0*	*0.0*	*0.0-1.0*	*97*	*26.7*	*19.6-28.1*	*171*	*41.7*	*36.9-46.6*
4	102	5	4.9	1.6-11.1	0	0.0	0.0-3.6	5	4.9	1.6-11.1	20	19.6	12.4-28.6
5	112	11	9.8	5.1-16.9	0	0.0	0.0-3.2	6	5.4	2.0-11.3	50	44.6	35.2-54.3
6	99	49	49.5	39.3-59.7	5	5.1	1.7-11.4	36	36.4	26.9-46.6	33	33.3	24.2-43.5
7	100	58	58.0	47.7-67.8	0	0.0	0.0-3.6	7	7.0	2.9-13.9	15	15.0	8.6-23.5
Voy 8	*413*	*123*	*29.8*	*25.4-34.4*	*5*	*1.2*	*0.4-2.8*	*54*	*13.1*	*10.0-16.7*	*118*	*28.6*	*24.3-33.2*
8a	25	23	92.0	74.0-99.0	6	24.0	9.4-45.1	0	0.0	0.0-13.7	7	28.0	12.1-49.4
8b	18	13	72.2	46.5-90.3	3	1.7	3.6-41.4	1	5.6	0.1-27.3	5	27.8	9.7-53.5
9	92	59	64.1	53.5-73.9	25	27.1	18.4-37.4	6	6.5	2.4-13.7	43	46.7	36.3-57.4
10	20	2	10.0	1.2-31.7	0	0.0	0.0-16.8	1	5.0	0.1-24.9	2	6.7	1.2-31.7
11	75	53	70.7	59.0-80.6	0	0.0	0.0-4.8	0	0.0	0.0-4.8	1	1.3	0.0-7.2
12a	143	99	69.2	61.0-76.7	11	7.7	3.9-13.3	8	5.6	2.4-10.7	19	13.3	8.2-20.0
12b	108	78	72.2	62.8-80.4	19	17.6	10.9-26.1	26	24.1	16.4-33.3	10	9.3	4.5-16.4
13	80	17	21.3	12.9-31.8	3	3.8	0.8-10.6	0	0.0	0.0-4.5	7	8.8	3.6-17.2
Voy 17	*561*	*344*	*61.3*	*57.1-65.4*	*67*	*11.9*	*9.4-14.9*	*42*	*7.5*	*5.4-10.0*	*94*	*16.8*	*13.8-20.1*
14	100	87	87.00	78.8-92.9	0	0.00	0.0-3.6	6	6.00	2.2-12.6	4	2.00	1.1-9.9
Total	*1,484*	*622*	*41.9*	*39.4-44.5*	*72*	*4.8*	*3.8-6.1*	*199*	*13.4*	*11.7-15.2*	*387*	*26.1*	*23.9-28.4*

BCoV was present in 92% of single viral detections and 92% of mixed viral-bacterial detections.

The individual animal prevalence of BCoV in our study was similar to that recently reported for feedlot cattle on the south coast of New South Wales with clinical BRD. Other studies have reported similar prevalence measures in clinically normal feedlot cattle. [33]

There is conflicting evidence of association between presence of coronavirus and respiratory disease in cattle. Some studies suggest an increased risk of respiratory disease in cattle that are shedding BoCV in nasal secretions. [34]

Our findings from necropsies conducted on voyages suggested that the presence of BoCV in nasal secretions was linked to an increased odds of death due to respiratory disease during the voyage. [35]

Other studies have not found an association. [36]

Serological testing for BCoV antibodies was not performed as part of the study reported here due to a lack of a commercially available test kit. Other studies suggest that animals that are shedding BoCV in nasal secretions may be likely to have low serologic titres against BoCV. Animals with low antibody titres and shedding virus may be more susceptible to infection with these viruses and therefore the animals in our study that were shedding BoCV may be considered at risk of developing some form of respiratory infection with this pathogen.

Bovine viral diarrhoea virus (BVDV) was detected in nasal swabs from 44 out of 1,484 (3%) animals, with the highest prevalence estimates occurring in a cohort of Western Australian carry-over cattle (12%) and south Australian slaughter cattle (10%). BVDV was detected in nasal swabs and lung samples from animals that died during voyages but was not significantly associated with fatal respiratory disease. [37]

The seroprevalence of BVDV in this study (56%; Table 38), was consistent with previous reports for Australian cattle with seroprevalence estimates ranging from 45 to 77% [38], and also with the “approximately 60%” reported for Western Australian live export cattle in 1985. [39]

Seroconversion during the feeding period has been linked to an increased likelihood of requiring treatment for BRD. [40]

Thirteen out of 334 animals were nasal swab positive and seronegative for BVDV, and 3 out of 334 animals were nasal swab positive and seropositive. These animals were either transiently infected and captured immediately prior to, or soon after, mounting an immune response, or they were persistently infected (PI). Persistently infected animals have viral antigen in their nasal secretions and are mostly seronegative.

[33] Hasoksuz et al. (2002); Fulton et al. (2011)
[34] Lathrop et al. (2000); Hasoksuz et al. (2002); Thomas et al. (2006); Fulton et al. (2011)
[35] Moore et al. (2014)
[36] Cho et al. (2001); Hasoksuz et al. (2005)
[37] Moore et al. (2014)
[38] Dunn et al. (1995); Taylor et al. (2006)
[39] Littlejohns and Horner (1990)
[40] O'Connor et al. (2001)

In feedlots PI animals can shed BVDV and infect susceptible contact animals (pen mates). Acute BVDV infection is well characterised in cattle and is known to impair immune cell function and predispose animals to secondary bacterial infections of the respiratory tract. PI animals therefore represent a threat to other healthy animals in the immediate area particularly if they have no immunity against BVDV.

Consideration should therefore be directed at detection and management of potential PI animals during the assembly period to minimise the risk of BVDV infection and subsequent development of BRD. It is also important to note that the application of a single PACE test to remove PI animals does not guarantee the removal of BVDV circulation from a live export consignment. [41]

Bovine herpesvirus 1 prevalence was similar to that observed in cattle at feedlot entry in the U.S. [42]

Bovine herpesvirus 1 is known to play an important role in the development of BRD through both direct tissue damage and immunosuppressive effects that allow secondary bacterial infections to cause respiratory disease. However, nasal shedding of BoHV-1 is not necessarily associated with respiratory disease or reduced performance in feedlot cattle. [43]

Results from voyage necropsies conducted as part of this project did not find an association between BoHV-1 in nasal or lung samples and mortality due to BRD. [44]

Thirty-nine percent (122/309) of newly received animals were seropositive for antibodies to BoHV-1. This is within the range of previously reported seroprevalences for Australian beef cattle (13-85%). [45]

The 61% of animals that were seronegative in our sampling are likely to be susceptible to infection by pen-mates shedding BoHV-1. No significant increase in BoHV-1 nasal prevalence was recorded for newly received animals sampled at feedlot entry and again 9 days later.

These findings suggested that BoHV-1 did not play a primary role in the development of BRD during voyages in the cattle we studied.

Bovine respiratory syncytial virus (BRSV) is recognised as a common primary pathogen in respiratory disease in young calves [46] and adult dairy cattle. [47]

BRSV has not previously been detected in nasal swabs from beef feedlot cattle over the age of 4 months. [48] In this study the significant increase in BRSV nasal prevalence between depot entry and re-resting 9 days later (cohorts 12a and 12b) is likely to indicate transmission of BRSV to naïve animals during this period.

[41] Moore et al. (2014)
[42] Fulton et al. (2002); Storz et al. (2000)
[43] Fulton et al. (2002)
[44] Moore et al. (2014)
[45] Dunn et al. (1995); Dunn et al. (2000)
[46] Sacco et al. (2013)
[47] Bidokhti et al. (2012)
[48] Moore (2014)

The seroprevalence of BRSV in the study reported here was 46%. This is higher than the 27% prevalence reported in a serosurvey of Australian feedlot cattle [49], and lower than the 100% reported in an outbreak of respiratory disease in New South Wales. [50]

The seroprevalence for BPIV-3 (87%) was the highest out of the four viruses. This, combined with a low nasal prevalence (1.4%) and lack of evidence for an association between BPIV-3 and respiratory disease in Australian live export cattle [51], or Australian feedlot cattle [52], suggests that BPIV-3 plays a secondary role, if any, in the development of BRD in Australian live export cattle.

Two of the respiratory bacteria were detected from every cohort and voyage with an overall combined prevalence of 42% for *Histophilus somni* and 26% for *Pasteurella multocida* while cohort prevalence estimates ranged from 1-92% (*H. somni*) and from 1-61% (*P. multocida*) (Table 29).

The overall prevalence for detection of *Mannheimia haemolytica* was 13%, there were four cohorts that had no *M. haemolytica* detected and the cohort level prevalence in those cohorts that did detect *M. haemolytica* ranged from 4.5-44%.

There was relatively little *Mycoplasma bovis* detected. The overall prevalence was 5%, 9 of 16 cohort-samples were completely negative and the prevalence in the seven cohort samples where *M. bovis* was detected ranged from 2-27%.

Previous studies examining the presence of bacteria in nasal swab samples have used bacterial culture for isolation and identification of bacteria. Our approach involved the use of PCR detection of bacterial DNA and did not involve bacterial culture. Bacterial culture is considered to be less sensitive than PCR when detecting presence of bacteria in swab samples. [53] PCR may also return a positive result even when there are no live or viable bacteria present because it is capable of detecting non-viable fragments of bacterial genome. Understanding some of the different characteristics of different methods is important when comparing our findings to those from other papers that may have used different techniques. [53]

Histophilus somni can be carried in the upper respiratory tract in normal, healthy animals without disease. *H. somni* is also a potential cause of a number of disease syndromes including fibrinopurulent pneumonia, myocarditis and polyarthritis-serositis. [54]

The individual animal and cohort level prevalence estimates for *H. somni* in the current project were both approximately 42%. This is higher than previous reports where individual animal prevalence ranged from 0-9%. [55]

Previous studies have not found a direct association between nasal isolation of *H. somni* at feedlot entry and risk of subsequent respiratory disease in feedlot cattle. [56]

[49] Dunn et al. (1995)
[50] Hick et al. (2012)
[51] Moore et al. (2014)
[52] Dunn et al. (1995); Dunn et al. (2000)
[53] Fulton and Confer (2012)
[54] Griffin et al. (2010)
[55] Allen et al. (1991); Corbeil et al. (1986); Fulton et al. (2002); Van Donkersgoed et al. (1994)
[56] Allen et al. (1991); Fulton et al. (2002)

Results from voyage necropsies conducted during this project found relatively high prevalence of *H. somni* detection in live export cattle that died during voyages but no association between detection of *H. somni* and either histological evidence of pneumonia or death due to BRD. [57]

The prevalence of *Mannheimia haemolytica* was 13% at the individual level and 10% at the cohort level. These findings were consistent with previously reported prevalence estimates for *M. haemolytica* in nasal swabs from clinically normal feedlot cattle.

As for other bacteria, *M. haemolytica* is found as a commensal organism of the nasopharynx and tonsils of healthy cattle, even though it is capable of causing respiratory disease under favourable conditions.

Pasteurella multocida was detected in nasal swabs from 26% of cattle with a cohort level prevalence of 27%. These findings were consistent with previously reported prevalence estimates for *P. multocida* in nasal swabs from clinically normal feedlot cattle. [58]

As for other bacteria under investigation in this project, *P. multocida* is found as a commensal organism of the nasopharynx and tonsils of healthy cattle, even though it is capable of causing respiratory disease under favourable conditions. [58] The presence of *P. multocida* in nasal swabs therefore does not necessarily mean increased risk of respiratory disease in those animals.

One study on lung tissue or swabs from beef cattle at necropsy collected between 1994 and 2002 detected a trend over that time period towards an increased isolation of *P. multocida* and reduced isolation of *M. haemolytica* as the principle bacterial pathogen associated with BRD. [59]

Our findings from necropsy samples collected form cattle that died during export voyages found that *P. multocida* was isolated more frequently than *M. haemolytica* in lung and nasal swab samples. [60]

There are a number of possible explanations for this trend including changes in bacterial virulence and antimicrobial resistance, changes in the efficacy of available vaccines and antibiotics, reduced age of cattle at feedlot entry, changes in the way sick cattle are identified and treated, and increased use of mass medication programs. [61]

Mycoplasma bovis (*M. bovis*) is an important cause of pneumonia, arthritis and tenosynovitis in feedlot cattle. [62]

M. bovis was detected in nasal swabs from 5% of cattle in our study. Previously papers have reported prevalence estimates of *M. bovis* in apparently healthy feedlot calves range from 0-43%. [63]

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[57] Moore et al. (2014)
[58] Fulton et al. (2002); Allen et al. (1991)
[59] Welsh et al. (2004)
[60] Moore et al. (2014)
[61] Rice et al. (2007); Welsh et al. (2004)
[62] Caswell et al. (2010)
[63] Allen et al. (1991); Hanzlicek et al. (2011); White et al. (2010); Wiggins et al. (2007)

The role of *M. bovis* in the development of BRD remains relatively undefined in the scientific literature. In our study, the findings from necropsy samples collected during the voyage suggested that *M. bovis* was significantly associated with mortality due to respiratory disease during voyages. [64]

Multiple organisms were detected in nasal swab samples from many animals in this study. Overall, a single bacteria only was detected in 503/1150 (43.7%) animals, while one or more viruses and bacteria were detected in 434 (37.7%) animals, and one or more viruses in 213 (18.5%) animals.

Spearman correlation coefficients were used to look for statistical associations between presence of multiple pathogens in the same swab sample.

The presence of BCoV was significantly correlated with the presence of BRSV, *M. haemolytica, P. multocida* and *H. somni* ($p<0.01$). Bovine viral diarrhoea virus was present in 8% of mixed infections but was not significantly correlated with any other viruses or bacteria ($p>0.05$). The presence of BRSV was significantly correlated with the presence of *H. somni* ($p<0.001$). Bovine herpesvirus 1, BVDV and BPIV-3 were not significantly correlated with any other organism.

Histophilus somni was present in 77% of single bacterial detections and 54% of mixed viral-bacterial detections. The presence of *H. somni* was significantly correlated with the presence of BCoV, BRSV, *M. bovis* ($p< 0.001$) and *P. multocida* ($p< 0.05$). *P. multocida* was present in 33% of single bacterial detections and 51% of mixed viral-bacterial detections. The presence of *P. multocida* was significantly correlated with the presence of BCoV ($p<0.001$), *M. haemolytica* $p<0.001$) and *H. somni* ($p<0.05$).

It is not surprising that co-infections were common given our understanding of the way that respiratory disease develops in cattle. Co-shedding of BCoV and BRSV has not been reported previously, to the best of our knowledge.

Two cohorts were each sampled twice to look for change in viral and bacterial detection over time (Cohort 8 and Cohort 12).

Cohort 8 represented animals that had been carried over in the assembly feedlot from the previous voyage preparation. The second sampling showed a reduction in prevalence or no change for all five viruses, but the change was not statistically significant for any of these comparisons.

Cohort 12 was sampled for the first time at the point of arrival at the assembly feedlot and then again several days later. There was no evidence of any significant change for four of the five viruses. The exception was for BRSV, where the second prevalence (3.7%) was significantly higher than the first prevalence (0.0%, $p=0.03$).

For bacterial pathogens there was no difference between repeat samples in Cohort 8 ($p>0.05$).

[64] Moore et al. (2014)

In Cohort 12, there was a significant increase in the prevalence of *M. bovis* (p=0.02) and *M. haemolytica* (p<0.001) over time while there was no difference in prevalence of *H. somni* (p>0.05) or *P. multocida* (p>0.05).

The increase in prevalence in repeated samples from the same group over time is likely to be due to the proliferation of commensal bacteria following inhibition of the immune system secondary to environmental stressors, for example transportation, co-mingling, and inter-animal transmission of viruses and bacteria. It is also possible that the results may simply reflect variation over time without any real underlying change in proliferation or carriage/infection rates.

Our results showed a rise in prevalence when the same group of animals was sampled at assembly depot entry and resampled nine days later (rise from 8% to 18%), suggesting that there was proliferation and / or spread of bacteria within and between animals.

8.4 Screening of explanatory factors against nasal prevalence

Negative binomial regression with adjustment for clustering at the level of the PIC group (representing each cohort) was used to analyse the data for possible associations between nasal prevalence and other explanatory factors.

Analyses were restricted to those cohorts that were tested for the first time only (excluding two cohort sets that were tested a second time) and those animals that were loaded onto a ship (excluding one cohort set was tested but was retained in the assembly feedlot and not loaded onto the ship).

Separate models were run for each pathogen with the outcome variable for each animal coded as zero=negative or one=positive for each specific pathogen based on the qPCR result from nasal swabs. Analyses were restricted to those organisms that had higher prevalence (BCoV, *H. somni*, *M. haemolytica* and *P. multocida*) mainly because models were unstable when run for other pathogens where prevalence was low.

Table 30 shows results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens and voyage number as the explanatory variable. Results are expressed as a count of animals tested on each voyage and the number testing positive for each pathogen. Incidence rates were generated for each voyage against a reference voyage. The reference voyage in each model was selected as the voyage with the lowest prevalence of pathogen detection.

Table 30: Results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens and voyage number as the explanatory variable. Adjusted for clustering at the property level (PIC) and reported as incidence rate (IR), standard error (se) and 95% confidence interval (CI) for the incidence rate. Pairwise comparisons between each level of the explanatory variable are reported as p-values. Analysis restricted to those animals that had valid PIC numbers.

Outcome	Expl variable	No. tested (N)	No. positive (n)	(%)	IR	se	95% CI		Pairwise comparisons (p-values)		
							Lower	Upper			
BCoV	Voyage								5 vs	8 vs	17 vs
	5	200	188	94.0	3.6	3.08	1.6	8.3			
	8	413	122	29.5	1.40	0.41	0.81	2.50	0.02		
	17	435	86	19.8	Ref				0.002	0.20	
	18	100	47	47.0	2.30	0.84	1.10	4.70	0.30	0.20	0.02
H. somni	Voyage										
	5	200	14	7.0	Ref						
	8	413	123	29.8	4.40	4.01	0.74	26.20	0.10		
	17	435	253	58.2	8.10	7.30	1.36	47.80	0.020	0.01	
	18	100	87	87.0	11.70	10.90	1.91	72.30	0.01	0.00	0.20
M. haemolytica	Voyage										
	5	200	88	44.0	14.80	7.80	5.30	41.50			
	8	413	54	13.1	4.60	2.20	1.80	11.80	0.00		
	17	435	15	3.4	Ref				<0.001	0.001	
	18	100	6	6.0	1.80	1.30	0.43	7.50	0.00	0.13	0.40
P. multocida	Voyage										
	5	200	121	60.5	14.04	11.90	2.70	73.60			
	8	413	118	28.6	6.5	5.1	1.4	30.6	0.048		
	17	435	79	18.2	4.50	3.60	0.93	21.50	0.01	0.20	
	18	100	4	4.0	Ref				0.00	0.02	0.06

There was significant variation between voyages with respect to the risk of detection of pathogens. Incidence rate measures provide a measure of the comparison of risk between different voyages. The comparison between voyages with the lowest prevalence of pathogen detection and the voyage with the highest prevalence of pathogen shedding can be appreciated through the IR estimates. For prevalence of *Mannheimia haemolytica*, the voyage with the highest prevalence had a 14.4-fold higher risk of detection of the organism compared to the voyage with the lowest prevalence.

Table 31 shows results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens and season as the explanatory variable.

Table 31: Results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens and season as the explanatory variable. Adjusted for clustering at the property level (PIC) and reported as incidence rate (IR), standard error (se) and 95% confidence interval (CI) for the incidence rate. Pairwise comparisons between each level of the explanatory variable are reported as p-values. Analysis restricted to those animals that had valid PIC numbers.

Outcome	Expl variable	No. tested (N)	No. positive (n)	(%)	IR	se	95% CI Lower	95% CI Upper	p-value
BCoV	**Season**								
	Summer	200	188	94.0	2.6	1.03	1.2	5.7	0.018
	Autumn	948	255	26.9	Ref				
H. somni	**Season**								
	Summer	200	14	7.0	Ref				
	Autumn	948	463	48.8	6.64	6.3	1.02	43.1	0.047
M. haemolytica	**Season**								
	Summer	200	88	44.0	5.40	2.10	2.50	11.60	<0.001
	Autumn	947	75	7.9	Ref				
P. multocida	**Season**								
	Summer	200	121	60.5	2.90	1.10	1.30	6.10	0.01
	Autumn	948	201	21.2	Ref				

There was a significant association between season and detection of each of the four pathogens analysed. For three of the pathogens (BCoV, *M. haemolytica* and *P. multocida*), the prevalence was higher in Summer compared to Autumn and for *H. somni*, the prevalence was higher in Autumn than in Summer.

Table 32 shows results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens, and year as the explanatory variable.

Table 32: Results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens and year as the explanatory variable. Adjusted for clustering at the property level (PIC) and reported as incidence rate (IR), standard error (se) and 95% confidence interval (CI) for the incidence rate. Pairwise comparisons between each level of the explanatory variable are reported as p-values. Analysis restricted to those animals that had valid PIC numbers.

Outcome	Expl. variable	No. tested (N)	No. positive (n)	(%)	IR	se	95% CI Lower	95% CI Upper	Pairwise comparisons p-value 2010 vs	Pairwise comparisons p-value 2011 vs
BCoV	**Year**									
	2010	200	188	94.0	2.7	1.1	1.2	6.1		
	2011	413	122	29.5	1.10	0.30	0.65	1.80	0.03	
	2012	535	133	24.9	Ref				0.02	0.80
H. somni	**Year**									
	2010	200	14	7.0	Ref					
	2011	413	123	29.8	4.40	4.00	0.75	26.10	0.1	
	2012	535	340	63.6	9.00	8.10	1.50	52.30	0.02	0.002
M. haemolytica	**Year**									
	2010	200	88	44.0	12.50	5.70	5.10	30.60		
	2011	413	54	13.1	3.90	1.60	1.80	8.60	0.001	
	2012	535	21	3.9	Ref				<0.001	0.001
P. multocida	**Year**									
	2010	200	121	60.5	3.90	1.70	1.70	9.00		
	2011	435	118	27.1	1.80	0.51	1.00	3.10	0.06	
	2012	535	83	15.5	Ref				0.002	0.05

There was significant association between year and risk of detection of each of the four pathogens.

For BCoV, *M. haemolytica* and *P. multocida*, 2010 had the highest prevalence, significantly higher than both other years.

For *H. somni,* the reverse appeared to be the case with the lowest prevalence in 2010 and with 2012 having the highest prevalence.

Table 33 shows results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens and assembly depot as the explanatory variable.

Table 33: Results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens, and assembly depot as the explanatory variable. Adjusted for clustering at the property level (PIC) and reported as incidence rate (IR), standard error (se) and 95% confidence interval (CI) for the incidence rate, p-value reported for overall effect only without pairwise comparisons. Analysis restricted to those animals that had valid PIC numbers.

Outcome	Expl. variable	No. tested (N)	No. positive (n)	(%)	IR	se	95% CI Lower	95% CI Upper	Pairwise comparisons p-value
BCoV	**Assembly depot**								**<0.001**
	1	100	47	47.0	30.6	35.2	3.2	292.5	
	2	112	2	1.8	Ref				
	3	100	6	6.0	3.3	4.4	0.3	43.4	
	4	299	244	81.6	41.2	46.9	4.4	383.6	
	5	248	38	15.3	10.4	12.1	1.1	101.4	
	6	75	7	9.3	7.6	9.2	0.7	82.3	
	7	102	58	56.9	35.0	40.8	3.6	343.0	
	8	112	41	36.6	20.1	23.3	2.1	194.6	
H. somni	**Assembly depot**								**0.053**
	1	100	87	87.0	21.4	30.3	1.3	344.3	
	2	112	11	9.8	2.4	3.8	0.1	52.9	
	3	100	58	58.0	12.5	17.9	0.8	207.4	
	4	299	63	21.1	9.5	13.5	0.6	152.6	
	5	248	139	56.0	15.8	22.4	1.0	252.0	
	6	75	53	70.7	15.3	21.7	0.9	249.1	
	7	102	5	4.9	Ref				
	8	112	61	54.5	13.8	19.6	0.8	224.4	
M. haemolytica	**Assembly depot**								**<0.001**
	1	100	6	6.0	2.5	2.3	0.4	15.2	
	2	112	6	5.4	2.3	2.2	0.3	14.9	
	3	100	7	7.0	1.6	1.7	0.2	13.0	
	4	299	124	41.5	16.8	12.5	3.9	72.0	
	5	248	8	3.2	Ref				
	6	75	0						
	7	102	5	4.9	2.8	2.8	0.4	20.1	
	8	112	7	6.3	2.7	2.4	0.5	15.7	
	Assembly depot								**<0.001**
P. multocida	1	100	7	7.0	Ref				
	2	112	50	44.6	9.9	7.5	2.3	43.6	
	3	100	15	15.0	2.8	2.4	0.5	14.7	
	4	299	154	51.5	9.4	6.9	2.3	39.4	
	5	248	33	13.3	3.6	2.8	0.8	16.1	
	6	75	1	1.3	0.1	0.3	0.0	9.0	
	7	102	20	19.6	4.4	3.7	0.8	23.0	
	8	112	45	40.2	8.7	6.5	2.0	37.7	

There was a significant association between assembly depot and pathogen detection for all four pathogens. Pairwise comparisons to compare each assembly depot to each other deport were not performed, partly because of the number of comparisons and also because it was sufficient to determine that there was significant variation without necessarily trying to determine specific prevalence estimates for specific depots. Associations at the assembly feedlot level may be due to cattle factors (individual animals or property level factors), local environment or climate factors, effect of season and year, and management.

Table 34 shows results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens and state as the explanatory variable.

Table 34: Results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens, and state where the assembly depot is located as the explanatory variable. Adjusted for clustering at the property level (PIC) and reported as incidence rate (IR), standard error (se) and 95% confidence interval (CI) for the incidence rate, p-value reported for overall effect only without pairwise comparisons. Analysis restricted to those animals that had valid PIC numbers.

Outcome	Expl variable	No. tested (N)	No. positive (n)	(%)	IR	se	95% CI Lower	95% CI Upper	Pairwise comparisons p-value	
BCoV	**State**								**SA vs**	**Vic vs**
	SA	112	41	36.6	1.8	1.5	0.36	9.11		
	Vic	102	58	56.9	1.01	0.50	0.36	2.81	0.5	
	WA	1270	344	27.1	Ref				0.9	0.4
H. somni	**State**									
	SA	112	61	54.5	14.6	25	0.5	421		
	Vic	102	5	4.9	Ref				0.1	
	WA	1270	411	32.4	13.70	23.10	0.50	374.00	0.9	0.1
M. haemolytica	**State**									
	SA	112	7	6.3	Ref					
	Vic	102	5	4.9	1.10	1.50	0.08	17.10	0.9	
	WA	1270	151	11.9	1.60	1.30	0.30	7.80	0.6	0.8
P. multocida	**State**									
	SA	112	45	40.2	1.90	1.70	0.32	11.40		
	Vic	102	20	19.6	Ref				0.5	
	WA	1270	257	20.2	1.00	0.90	0.20	5.50	0.2	0.9

There was no association between state where the assembly depot was located and prevalence of any of the four pathogens of interest (p>0.05).

Table 35 shows results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens, and animal sex as the explanatory variable.

Table 35: Results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens, and animal sex (bull, heifer, steer) as the explanatory variable. Adjusted for clustering at the property level (PIC) and reported as incidence rate (IR), standard error (se) and 95% confidence interval (CI) for the incidence rate, p-value reported for overall effect only without pairwise comparisons. Analysis restricted to those animals that had valid PIC numbers.

Outcome	Expl. variable	No. tested (N)	No. positive (n)	(%)	IR	se	95% CI Lower	95% CI Upper	Pairwise comparisons p-value	
BCoV	**Sex**								**Bull vs**	**Heifer vs**
	Bull	307	79	25.7	Ref					
	Heifer	172	49	28.5	1.2	0.5	0.46	2.92	0.8	
	Steer	649	315	48.5	1.00	0.23	0.65	1.60	0.9	0.8
H. somni	**Sex**									
	Bull	307	200	65.1	1.5	0.3	0.94	2.27		
	Heifer	172	90	52.3	1.50	0.60	0.70	3.13	0.9	
	Steer	649	185	28.5	Ref				0.10	0.300
M. haemolytica	**Sex**									
	Bull	307	24	7.8	1.64	1.30	0.34	7.90		
	Heifer	172	12	7.0	Ref				0.5	
	Steer	649	126	19.4	2.40	1.80	0.50	10.60	0.4	0.3
P. multocida	**Sex**									
	Bull	307	71	23.1	1.20	0.60	0.40	3.20		
	Heifer	172	41	23.8	Ref				0.8	
	Steer	649	208	32.0	1.40	0.70	0.60	3.80	0.5	0.4

There was no association between animal sex and prevalence of any of the four pathogens ($p>0.05$).

Table 36 shows results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens, and animal type as the explanatory variable.

Table 36: Results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens, and animal type (*Bos indicus* vs *Bos taurus*) as the explanatory variable. Adjusted for clustering at the property level (PIC) and reported as incidence rate (IR), standard error (se) and 95% confidence interval (CI) for the incidence rate, p-value reported for overall effect only without pairwise comparisons. Analysis restricted to those animals that had valid PIC numbers.

Outcome	Expl variable	No. tested	No. positive		IR	se	95% CI		p-value
		(N)	(n)	(%)			Lower	Upper	
BCoV	**Type**								
	indicus	172	39	22.7	1.3	0.25	0.9	1.9	0.2
	taurus	976	404	41.4	Ref				
H. somni	**Type**								
	indicus	172	98	57.0	1.02	0.2	0.72	1.5	0.9
	taurus	976	379	38.8	Ref				
M. haemolytica	**Type**								
	indicus	172	20	11.6	1.10	0.40	0.60	2.20	0.8
	taurus	976	143	14.7	Ref				
P. multocida	**Type**								
	indicus	172	40	23.3	1.10	0.30	0.70	1.90	0.6
	taurus	976	282	28.9	Ref				

There was no association between animal type (*indicus* vs *taurus*) and detection of pathogens (p>0.05).

Table 37 shows results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens and animal class as the explanatory variable.

Table 37: Results from separate negative binomial models with pathogen detection (0=negative, 1=positive) as the outcome for each of four pathogens and animal class (breeder, feeder, slaughter) as the explanatory variable. Adjusted for clustering at the property level (PIC) and reported as incidence rate (IR), standard error (se) and 95% confidence interval (CI) for the incidence rate, p-value reported for overall effect only without pairwise comparisons. Analysis restricted to those animals that had valid PIC numbers.

Outcome	Expl variable	No. tested (N)	No. positive (n)	(%)	IR	se	95% CI Lower	95% CI Upper	Pairwise comparisons p-value Br. vs	Pairwise comparisons p-value Fe. Vs
BCoV	**Class**								**Br. vs**	**Fe. Vs**
	Breeder	100	47	47.0	1.8	1	0.6	5.2		
	Feeder	749	330	44.1	1.00	0.40	0.50	2.20	0.3	
	Slaughter	299	66	22.1	Ref				0.3	0.9
H. somni	**Class**									
	Breeder	100	87	87.0	2	0.9	0.8	4.7		
	Feeder	749	264	35.2	1.10	0.40	0.60	2.10	0.2	
	Slaughter	299	126	42.1	Ref				0.1	0.8
M. haemolytica	**Class**									
	Breeder	100	6	6.0	Ref					
	Feeder	749	113	15.1	2.00	2.20	0.20	17.70	0.6	
	Slaughter	299	44	14.7	3.10	3.50	0.30	28.40	0.3	0.4
P. multocida	**Class**									
	Breeder	100	4	4.0	Ref					
	Feeder	749	261	34.8	6.70	6.50	1.00	44.50	0.048	
	Slaughter	299	57	19.1	4.80	4.80	0.70	33.40	0.1	0.3

There was a single significant comparison, involving feeder animals having a higher prevalence of *P. multocida* compared to breeder animals. There were only a relatively small sample of breeder animals in the study and these results should be viewed with some caution. All other comparisons between animal class levels were not significant ($p>0.05$).

Body weight was recorded for 878 animals from eleven cohorts. In ten cohorts, body weight measures were recorded from almost all of the animals that were sampled. In the remaining cohort, body weight was recorded for a single animal out of a cohort total of 102 animals. An attempt was made to screen for an association between body weight, as a continuous variable (kg liveweight) and disease prevalence. Because there was an association between cohort and disease prevalence and body weight was only available for some cohorts, there was some risk of inadvertent bias. The analysis for association with body weight was done by using a general linear model with weight in kg as the continuous outcome and disease prevalence as the predictor. Models were adjusted for clustering using the same PIC code as was used for other screening.

Figure 7 shows the mean body weight by class and the 95% confidence interval for the mean. Feeder animals had the lowest mean body weight (265 kg), followed by breeders (306 kg) and slaughter animals (427 kg).

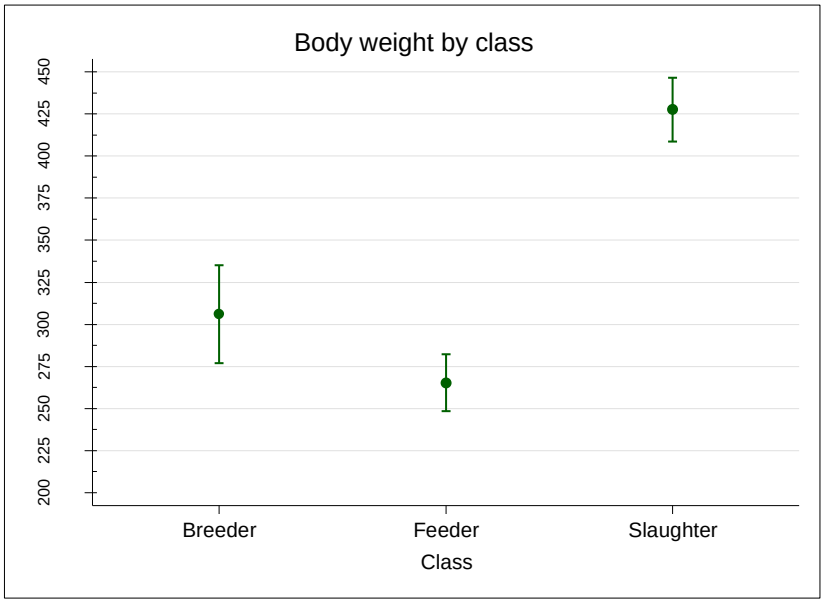


Figure 7: Summary of body weight by class. Bars represent 95% confidence intervals.

Figure 8 shows the mean body weight by sex. Heifers had the lowest mean body weight (283 kg) followed by steers (343 kg) and bulls (351 kg).

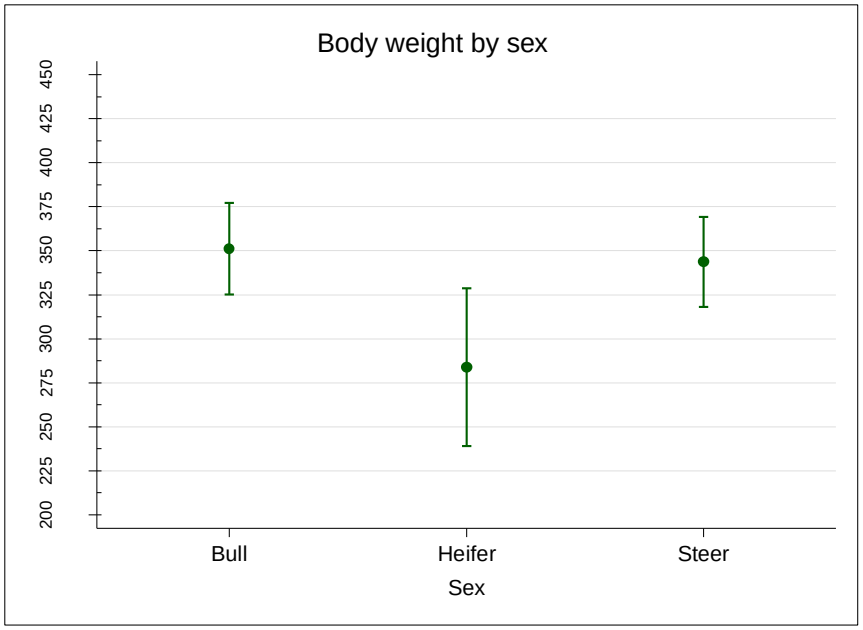


Figure 8: Summary of body weight by sex. Bars represent 95% confidence intervals.

There was no association between body weight and BCoV prevalence (p=0.9), *H. somni* prevalence (p=0.4), *M. haemolytica* prevalence (p=0.2), or *P. multocida* prevalence (p=0.6).

Of the epidemiological factors for which data was available, property of origin appeared to have the most significant association with both nasal and seroprevalence. When differences in nasal prevalence between properties was taken into account, no significant differences were detected between animals from different states or of different sex, type (*Bos indicus* vs *Bos taurus*), class (feeder, slaughter, breeder), or weight categories.

Co-mingling of cattle from multiple sources has been linked to an increased incidence of BRD in feedlot cattle. [65] Co-mingling is considered likely to be a source of stress and exposure of animals of unknown health status to a variety of viral and bacterial organisms.

Under Australian export conditions, the majority of animals on long-haul voyages are sourced directly from their property of origin to meet import protocol requirements. The first opportunity for co-mingling in the export supply chain therefore occurs as animals arrive at the assembly depot. The wide range in nasal and sero-prevalence between properties does support the hypothesis that there may be substantial opportunity for spread of pathogens from groups with higher prevalence to groups with lower prevalence once they are co-mingled in the assembly depot. This is consistent with our findings (see Section 8.3) of a rise in pathogen prevalence from first to second sampling in groups of animals that were sampled twice at different time periods.

8.5 Seroprevalence

Serum samples were collected from 334 animals distributed across four cohort groups, all from one voyage.

Table 38 shows summary information on seroprevalence results by sampling cohort. Each row presents summary information from one cohort and displays a count of the number of animals tested, the number and % that tested positive (prevalence) and the 95% confidence interval for the prevalence.

[65] Sanderson, Dargatz, and Wagner (2008; Taylor et al. (2010)

Table 38: Summary of seroprevalence results presented by virus and cohort group. Prevalence presented as a percentage of the animals tested in each cohort and with 95% confidence intervals (CI).

					Prevalence	95% CI	
	Cohort	No. tested	No. with conclusive results	No. positive	% of tested	Lower	Upper
		n	n	n	%		
BoHV-1	8a	25	25	23	92.0	75.0	97.8
	9	91	85	49	53.8	43.7	63.7
	11	75	74	59	78.7	68.1	86.4
	12a	143	140	14	9.8	5.9	15.8
Subtotal all		*334*	*324*	*145*	*43.4*	*38.2*	*48.8*
Subtotal new arrivals		*309*	*299*	*122*	*39.5*	*34.2*	*45.0*
BRSV	8a	25	25	25	100.0	86.7	100.0
	9	91	90	34	37.4	28.1	47.6
	11	75	75	10	13.3	7.4	22.8
	12a	143	143	98	68.5	60.5	75.6
Subtotal all		*334*	*333*	*167*	*50.0*	*38.2*	*48.8*
Subtotal new arrivals		*309*	*308*	*142*	*46.0*	*40.5*	*51.5*
BVDV	8a	25	25	21	84.0	65.3	93.6
	9	91	84	51	56.0	45.8	65.8
	11	75	71	54	72.0	61.0	80.9
	12a	143	142	67	46.9	38.9	55.0
Subtotal all		*334*	*322*	*193*	*57.8*	*38.2*	*48.8*
Subtotal new arrivals		*309*	*297*	*172*	*55.7*	*50.1*	*61.1*
BPIV-3	8a	25	24	24	96.0	80.5	99.3
	9	91	91	72	79.1	69.7	86.2
	11	75	75	59	78.7	68.1	86.4
	12a	143	143	139	97.2	93.0	98.9
Subtotal all		*334*	*333*	*294*	*88.0*	*38.2*	*48.8*
Subtotal new arrivals		*309*	*309*	*270*	*87.4*	*83.2*	*90.6*

Cohort 8a represented animals that had been carried over in the assembly feedlot from the previous voyage preparation. There was interest in whether carry-over animals may have increased opportunity to be exposed to circulating pathogens because of the additional time they spend in the feedlot. If this was the case then carry-over animals might be expected to have a higher seroprevalence than animals that were entering the assembly feedlot for the first time.

Chi-squared tests were used to compare the prevalence in carry-over animals vs all other cohorts combined. Carry-over animals had a higher prevalence of BoHV-1 ($p<0.001$), BRSV ($p<0.001$), and BVDV ($p<0.001$) but there was no difference in seroprevalence of BPIV-3 ($p=0.17$).

When individual animal seroprevalence status results were assessed, animals were more likely to be seropositive to multiple viruses based on testing of the same sample than to be seropositive to a single virus and negative to all other viruses that were tested. These findings indicate that it is very common for multiple viruses to be circulating at the same time and for animals to be exposed to multiple viruses at once.

Animals that were tested on arrival at the assembly depot for the first time (avoiding carry over animals) and that had both serological test results and nasal swab qPCR results available from the same animals, were aggregated for comparison of serology and qPCR results (Table 39).

Table 39: Summary results for those animals sampled on entry to assembly feedlot (omitting carry over animals) and tested by both serological test (negative or positive) and nasal swab. Results presented as percentage of animals tested.

	Serological and nasal shedding status			
Serostatus	**negative**	**negative**	**positive**	**positive**
Nasal swab status	**positive**	**negative**	**positive**	**negative**
Virus	**Percentage of animals with serum & nasal results**			
	(%)	**(%)**	**(%)**	**(%)**
BoHV-1	0.7	58.5	1.3	39.5
BRSV	0	53.9	0	46.1
BVDV	3.4	38.7	1	56.9
BPIV-3	2.6	10	0.6	86.7

Animals were most likely to be either negative on both serology and qPCR – indicating they had not been previously exposed and were not shedding virus – or they were seropositive and qPCR-negative indicating that they had been previously exposed and had developed an antibody response. Animals were believed to have not been vaccinated against respiratory disease pathogens prior to being sampled.

A small percentage of animals were seropositive and qPCR positive which may be consistent with animals that had been previously exposed and were nasal carriers, or they were seronegative and qPCR positive which may indicate animals that had either recently been exposed or were carriers.

Animals in the carry-over cohort had a higher percentage that were seronegative and qPCR-positive (3 from 25 or 12%) which may reflect increased opportunity for exposure with increased time in the feedlot.

9 Results - Retrospective data on voyage mortality

Retrospective data for sea voyages between January 1995 and December 2012 was obtained from the Shipboard Mortality Database (SMDB). Between January 1995 and December 2012, cattle were transported by sea from 29 ports in Australia to 124 ports in 30 countries around the globe. South East Asia accounted for the majority of exported cattle,

followed by MENA and NE Asia. The number of cattle exported to each market varies each year (Figure 9).

Table 40: Summary statistics for all voyages from Australia to all destinations between 1995-2012, MENA=Middle East and North Africa, SE= south east, NE= north east, Misc = miscellaneous.

Parameters	Units	MENA	SE Asia	NE Asia	SE Europe	Misc	Total
Voyages	N	1,028	4,909	395	14	101	6,447
Cattle loaded	N	2,632,296	9,378,399	700,567	75,170	198,084	12,984,516
Mortality overall	% of cattle loaded	0.44	0.09	0.12	0.28	0.46	0.17
Voyage mortality range		0-41.5	0-4.8	0-2.6	0-0.87	0-74.7	0-74.7
Average voyage duration	days	17.5	6.6	17	28.8	18.6	9.2
Average discharge period	days	3.8	0.9	1.3	3.6	1.4	1.4
Voyages with zero mortalities	N	293	2,533	118	1	31	2,976

Across all voyages the average mortality percentage (number of cattle dead/number of cattle loaded) was 0.17%. On 2,976 out of 6,447 voyages (46.2%) there were no mortalities.

The percentage mortality reported for voyages to MENA provides a long-term estimate based on a similar destination group as the twenty voyages enrolled in this project and described in more detail in Section 7.3. The overall percentage mortality reported in Table 40 for MENA (0.44%) was higher than the percentage mortality reported for the twenty voyages enrolled in this study (0.38%).

Figure 9 shows total numbers of cattle exported from Australia by year and destination region.

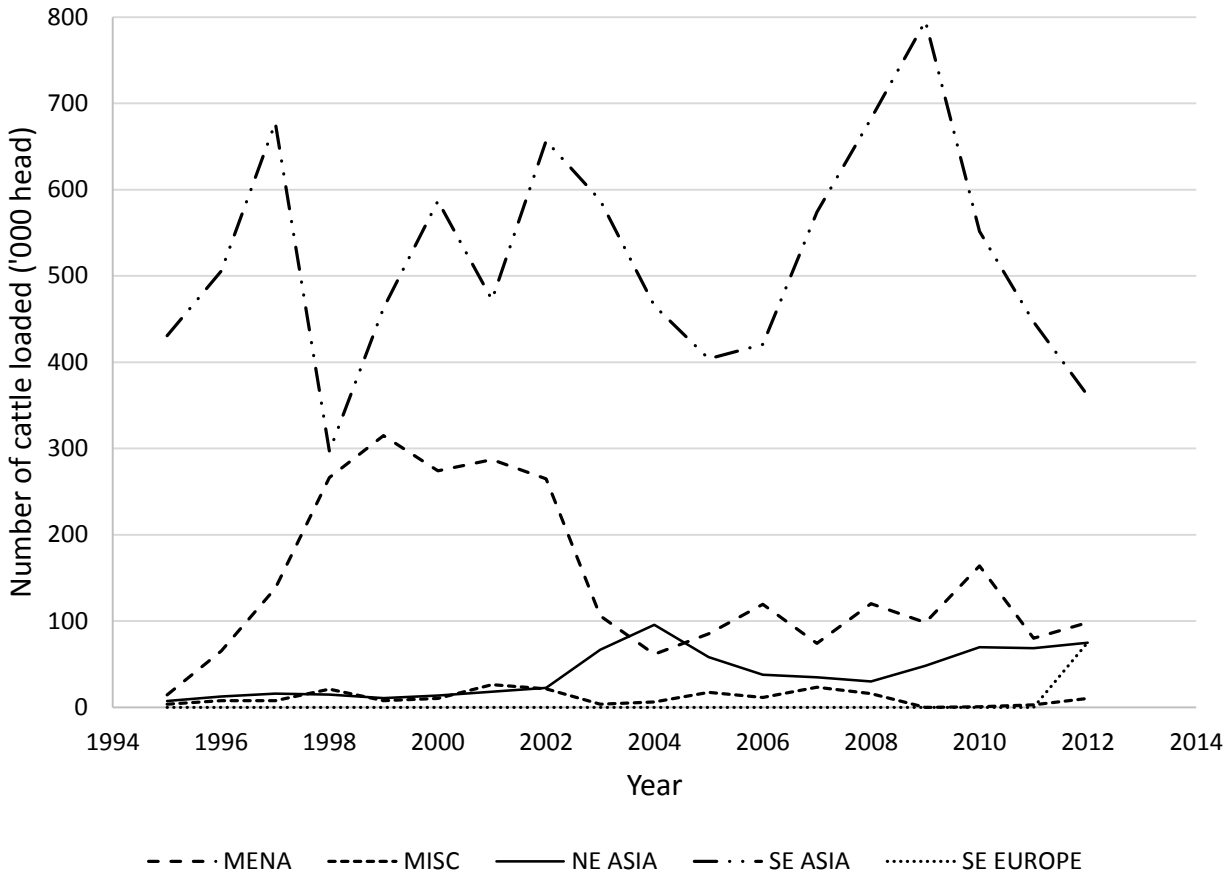


Figure 9: Total numbers of cattle exported from Australia by year and destination region between 1995 and 2012. Includes all data. Sourced from the Shipboard Mortality Database (SMDB).

Preliminary analyses of the SMDB data indicated that there were four voyages that had mortality percentages that were notably above the mortality percentages for all other voyages. Brief descriptive information from these four voyages is presented here:

- 1998: 346 voyage deaths on a voyage to Jordan on the “MV Charolais Express”, as a result of a heat stress event and inadequate ventilation.
- 1999: 830 cattle suffocated when power loss caused ventilation failure on the “Temburong” in January 1999.
- 1999: 191 cattle died on the “Kalymnian Express” in December 1999.
- 2002: 127 cattle died on the “MV Becrux”, as a result of temperatures and humidity in the Arabian Gulf.

These voyages all had mortality investigations completed and were all described as being special events associated with a combination of vessel mishaps (ventilation breakdown) and extreme weather conditions. The voyage mortality percentage for these four voyages was more than two-fold and up to nine times higher than the next highest voyage mortality percentage estimates from the entire dataset. In the years in which these voyages occurred,

the mean mortality estimate and 95% confidence intervals were meaningfully different in appearance when these voyages were either included or removed. A decision was made to remove these four voyages from the dataset for all subsequent modelling conducted in this section. The reason for this was because these voyages were extreme events and were not considered likely to represent long term trends or overall population patterns which were important objectives for this section.

Once these four voyages were removed the dataset used for analyses included 6,443 voyages.

9.1 Mortality rate

Summary statistics were generated from 6,443 voyages over the period 1995-2012 (excluding the four extreme voyages identified in exploratory screening). See Figure 11 for a graph showing mortality rate by year.

The overall average percentage mortality (deaths as a percentage of total cattle loaded) was 0.15% and when expressed as a rate, 0.13 deaths per 1,000 cattle-days at sea (across the period 1995-2012).

When the time period was restricted to 2010-2012, the average percentage mortality was 0.1% and the mortality rate overall was 0.08 deaths per 1,000 cattle-days at sea.

As reported in Section 7 of this report, the average voyage mortality rate for the twenty voyages enrolled in the detailed mortality study component of this study was 0.138 deaths per 1,000 cattle-days (95% confidence interval from 0.128–0.148).

A recent survey of Australian feedlots described mortality rates in Australian feedlot cattle based on electronic data records from several feedlots in eastern Australia over a 12 month period (2010-2011). [66] The dataset compiled for that study comprised more than 2,000 deaths and over 25 million cattle-days at risk and the study provides a representative estimate of mortality risk in land based feedlots. Mortality data from that study was compiled for those animals being held in feedlots for shorter periods (up to 120 days), representing the bulk of the mainstream domestic feedlot trade. The mortality rate (deaths per 1,000 cattle-days at risk) for feedlot cattle for all causes of death combined was 0.1 deaths per 1,000 cattle-days and for deaths due to respiratory disease was 0.06 deaths per 1,000 cattle-days at risk.

The findings from this study suggest that contemporaneous comparison of mortality rates in land based feedlots and export voyages have produced very similar estimates of mortality rate expressed as deaths per 1,000 days.

9.2 Voyage duration

Figure 10 shows a box and whisker plot summary of voyage duration for the different regions. Voyage duration includes all days until the last cattle were unloaded at the final destination port. In some cases, where voyages unloaded at multiple destination ports, the discharge days (days from first destination port to final destination port) were longer in duration than the voyage days (days from Australian load port to first destination port).

[66] Perkins (2013)

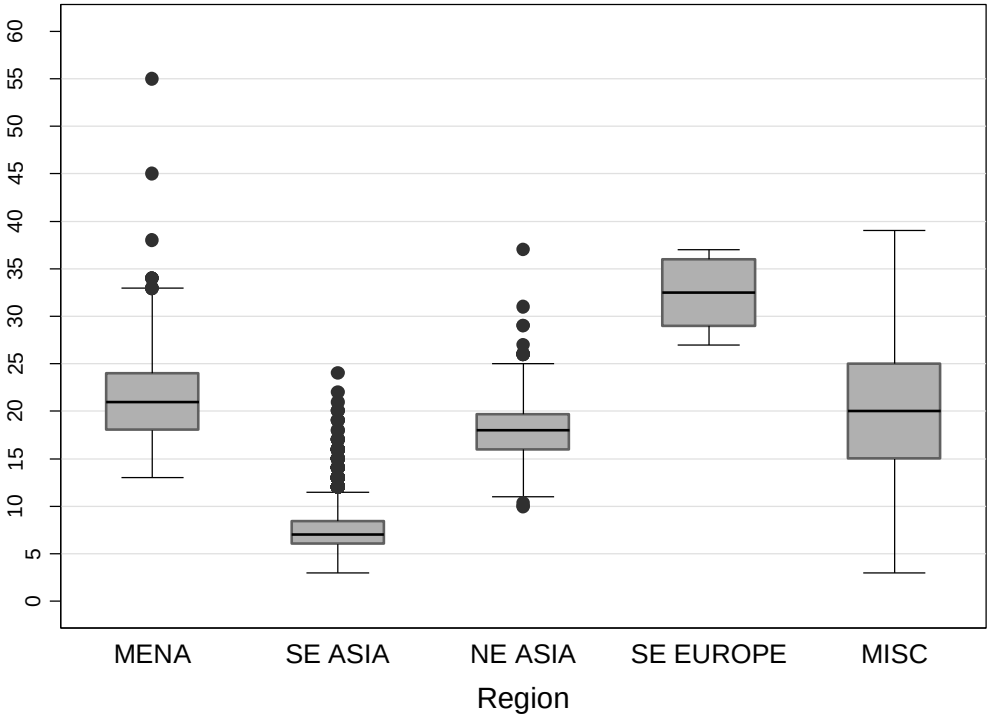


Figure 10: Box and whisker plot showing summary statistics for voyage duration in days (including sea voyage and discharge periods), based on data from voyages between 1995-2012.

The central shaded box spans the range from the 25th percentile to the 75th percentile and the horizontal line within the central shaded box is the median duration. The outer whiskers (lines) above and below the box, span out to a limit that is 1.5*IQR where the IQR is the inter-quartile range (the range from the 25th percentile to the 75th percentile). Any points that lie outside this range are plotted as shaded circles.

The box plots provide an excellent visual summary of voyage duration. The longest voyages based on median voyage duration were to SE Europe and the shortest voyages to SE Asia. There was a wide range of voyage durations within the MENA category including individual voyages with the longest voyage duration (voyage from Karumba on the QLD coast to Egypt).

9.3 Effect of year

Figure 11 shows annual mortality expressed as a percentage of cattle loaded for 6,443 voyages over the period 1995-2012 (excluding the four extreme voyages identified in exploratory screening).

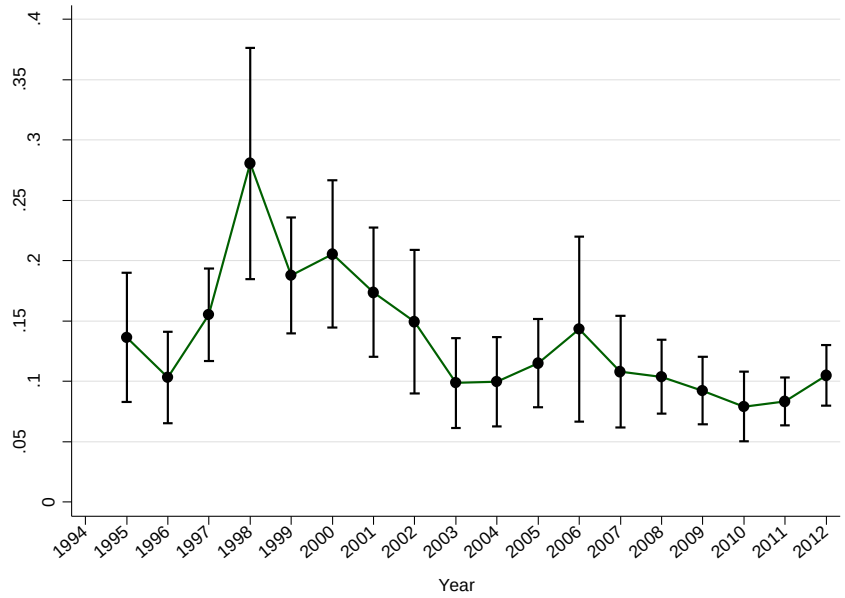


Figure 11: Mortality expressed as a percentage of cattle loaded per year from 6443 voyages over the period 1995-2012. Bars represent 95% confidence intervals.

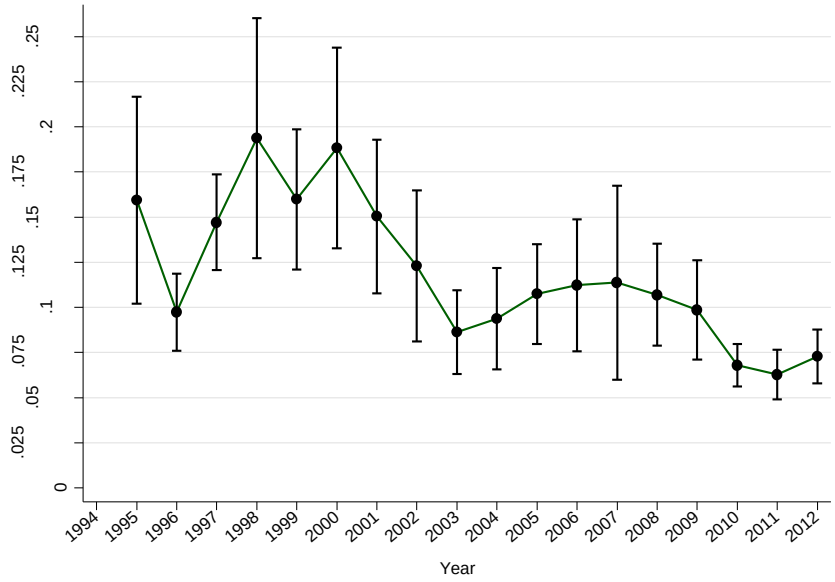


Figure 12: Mortality expressed as a mortality rate (deaths per 1,000 cattle-days) from 6,443 voyages over the period 1995-2012. Bars represent 95% confidence intervals.

Figure 12 shows annual mortality rate expressed as deaths per 1,000 cattle-days (incorporating adjustment for duration of voyage in days), for the same 6,443 voyages over the period 1995-2012.

Figure 11 and Figure 12 provide examples of two different approaches to estimation of mortality during export voyages, one based on percentage of cattle loaded, without any adjustment for voyage duration and the other based on a true mortality rate that incorporates adjustment for voyage duration (see Section 3.9 for more discussion on these two approaches).

Mortality percentage measures may make shorter voyages appear to have an artificially lower mortality and longer voyages an artificially higher mortality than the true underlying mortality risk when expressed in rate units (deaths per standardised count of cattle per day or per measure of cattle-days). This risk of bias means that simple percentage measures should not be used to compare mortality risk when voyages are very different in duration.

Most industry reports for mortality during export involve mortality expressed as a percentage of animals loaded.

The mortality rate as displayed in Figure 12, provides a measure that can be used to compare mortality rates between different voyages and also when analysing aggregated data from different voyages to assess effects of other explanatory factors (voyage, years etc). Mortality rates expressed as deaths per unit time at risk also allow comparison between different sources of mortality estimates, such as export vs on-farm vs on-feedlot. For this reason, we have used the mortality rate measure in analyses described in this report.

There was increasing variability in estimates prior to about 2003, as indicated by the wider confidence interval bars, and more variable annual mean estimates. There were peaks in mortality rate in 1998 and 2000 and then a progressive decline to levels that plateaued between 2003 and 2009 and then a further decline to a lower plateau with very tight confidence intervals for 2010-2012.

Pairwise comparisons between years were compared using FDR-adjusted p-values and in conjunction with visual appraisal of the general patterns apparent in Figure 12.

There was no statistical difference between annual mean mortality for 2001 compared to 2000 ($p=0.3$) or for 2002 compared to 2000 ($p=0.1$), however the mean for 2003 was significantly lower than 2000 ($p<0.001$). With the exception of 2007 ($p=0.4$), all of the years from 2003-2012 were significantly lower than 2000 ($p<0.05$).

There was no difference in mortality rate between 2003-2009 ($p>0.05$), suggesting that this was a statistical plateau in annual mortality rates.

There was then a visually apparent fall in annual mortality rate from 2009 to 2010 when mean mortality rate fell below 0.075 deaths per 1,000 cattle-days. There was a tendency for a difference from 2009 to 2010 ($p=0.063$) and the 2011 rate was significantly lower than 2009 ($p=0.046$).

For selected additional analyses, a year-grouping was developed by creating three levels: 1995-2002, 2003-2009 and 2010-2012.

The pattern in mortality rate over time may be related to broader chronological events. The Australian Livestock Export Standards (ALES) were developed in 1996-97 and implemented in 1998-99 by the Australian Livestock Export Corporation (LiveCorp). In 1999 and 2002, there were reviews of the livestock export trade by an Independent Reference Group (IRG) convened by the Federal Minister for Agriculture, Fisheries and Forestry. Both reviews were convened in response to concerns over incidents relating to live exports and whether there were appropriate controls in place to prevent such incidents in the future. In 2003, the Keniry Livestock Export Review [67] (Keniry 2003) was initiated in response to welfare concerns

[67] Keniry (2003)

arising from issues associated with the “MV Cormo Express”, an export vessel carrying sheep to Saudi Arabia that spent 80 days on the vessel following rejection of the consignment by Saudi authorities.

Significant reforms were made to industry regulation following the Keniry Livestock Export Review, which led to government taking on full responsibility for managing the regulation of the livestock export process. This included the development of the Australian Standards for the Export of Livestock (ASEL), which initially came into effect in July 2005. Since that time there have been several revisions of ASEL and the current version 2.3 of ASEL was endorsed in April 2011.

Over the recent decade, there have been a variety of changes in the way livestock exports are conducted, driven by a combination of market forces (changes in demand and supply of livestock) and regulatory requirements with associated changes in selection and management of animals.

9.4 Effect of month of year

Preliminary exploration of mortality estimates by month of year suggested that the pattern had changed over time, consistent with the general pattern of a reduction in mortality rate per annum over time. A decision was made to summarise monthly mortality rates using three broad categories of time based on the year groupings presented in the previous section.

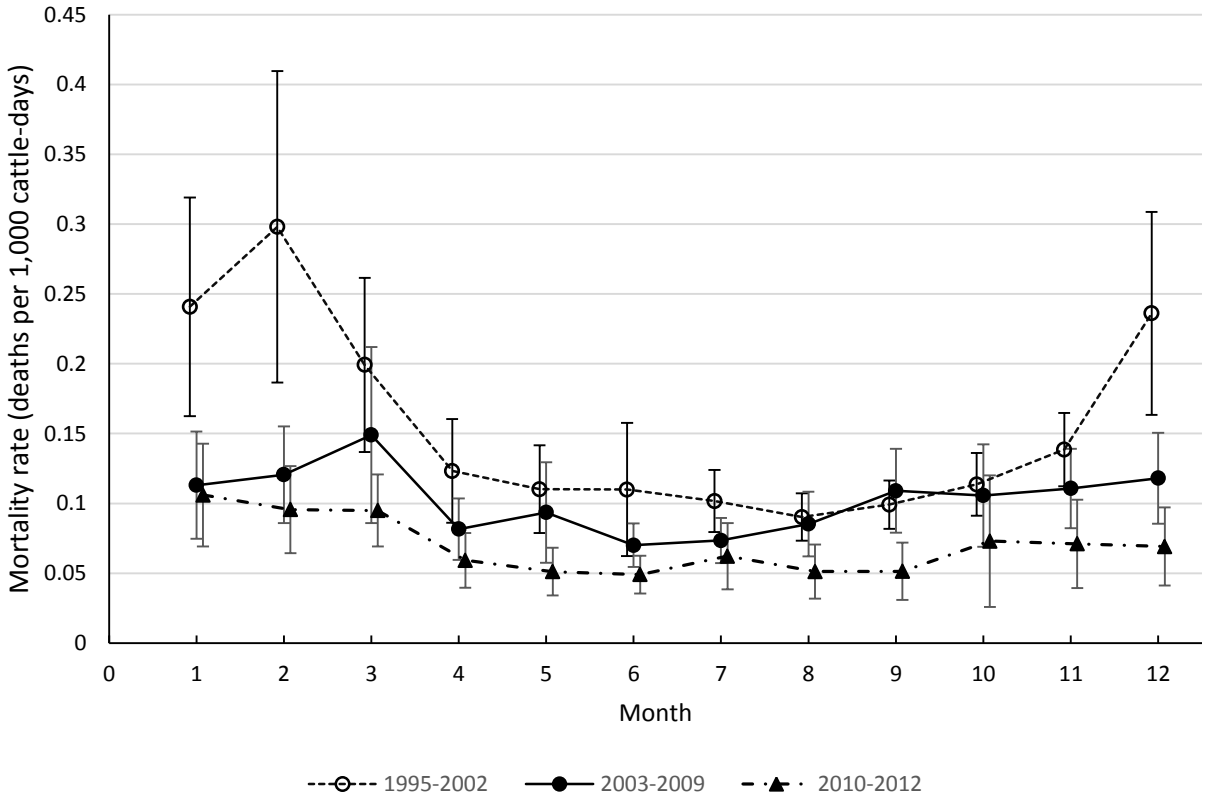


Figure 13: Mean monthly mortality rate (deaths per 1,000 cattle-days) arranged by three year-groups: 1995-2002, 2003-2009, 2010-2012. Bars represent 95% confidence intervals. Points for each year group have been jittered slightly at each month, so that confidence intervals do not overlap.

Each of the three year group categories shows a similar general pattern with a rise in monthly mortality rates during the Australian Summer months and a nadir in the Winter. There is also an overarching pattern due to the effect of year group. In the earliest year group (1995-2002), the Summer rise in monthly mortality rate was higher than for other year groups. The lower monthly values through the Winter also remained higher than the monthly estimates for the later year groups.

Selected statistical comparisons were performed to further explore the effects of month of year within the different year-groups (1995-2002; 2003-2009; 2010-2012).

Within the 1995-2002 year-group, there was a significant reduction in mortality rate from March to April ($p=0.016$). Between March and August there was no significant difference in mortality rate. Then there was a progressive rise in mortality rate from August onwards. The change from August to November was significant ($p=0.001$) and there was a further significant rise from November to December ($p=0.003$). There was then no difference in mortality rates between any of the months from December to March.

A similar pattern was evident in the 2003-2009 year-group, however it was less obvious. The peak mortality rate in March was followed by a significant fall to April ($p=0.014$) and then a plateau through the Winter with a nadir in July, followed by a progressive rise in the second half of the year. The rise from July to September was significant ($p=0.013$) and then there was no difference between months from September through to March.

Within the 2010-2012 year-group the mortality rates followed a similar pattern again, however the extremes had been further reduced. There was a peak mortality in January that was followed by a progressive decline in monthly mortality rate. The reduction became statistically significant from March to April ($p=0.04$) and then mortality rate remained low through the Winter. The lowest monthly mortality was in June and there was then little rise until October.

The progressive month to month rise late in the year was small and not statistically significant. Comparing the low values in June or September to the peak in January produced a statistically significant increase ($p<0.05$).

Comparisons were also performed between year-groups at each month. The most recent year-group (2010-2012) was significantly lower than 1995-2002 at all months, except October ($p<0.05$).

The 2003-2009 year-group was significantly lower than the 1995-2002 group in January, February, April, July and December ($p<0.05$).

The 2010-2012 year-group was significantly lower than the 2003-2009 group in May, June, August, September and December ($p<0.05$).

In conclusion, there is a general seasonal pattern with a peak mortality for voyages occurring in Summer months that persists through until March. There is then a progressive decline to a nadir in Winter, that is followed by a progressive rise through Spring and Summer to the annual peak.

While the pattern has remained pretty constant through the 20 year period covered by the dataset, there has been a general and progressive reduction in mortality rates over that

period. The most prominent reduction from earlier time periods (1995-2002) to later periods is a reduction in Summer month peak mortality rates. The lowest mortality rates in every month were achieved in the most recent year-group (2010-2012). The evidence is consistent with a progressive reduction in monthly mortality rates over time, with the biggest effect appearing in those Summer months that had the highest mortality rates in the 1990's.

9.5 Effect of destination region

Countries included in each region were defined as follows:

- MENA (Middle East and North Africa): Bahrain, Egypt, Israel, Jordan, Kuwait, Libya, Oman, Pakistan, Qatar, Saudi Arabia, Sudan, United Arab Emirates;
- SE Asia (South East Asia): Brunei, Indonesia, Malaysia, Philippines, Singapore, Vietnam;
- NE Asia (North East Asia): China, Japan, Russian ports on the Pacific facing coast of Russia, South Korea;
- SE Europe (South East Europe): Turkey and Russian ports on the Black Sea; and
- MISC (Miscellaneous): East Timor, Mauritius, Mexico, New Caledonia, Russian ports on the west coast near Finland, Samoa, Solomon Islands and Sri Lanka.

Summary statistics for voyages by region were presented. Retrospective data for sea voyages between January 1995 and December 2012 was obtained from the Shipboard Mortality Database (SMDB). Between January 1995 and December 2012, cattle were transported by sea from 29 ports in Australia to 124 ports in 30 countries around the globe. South East Asia accounted for the majority of exported cattle, followed by MENA and NE Asia. The number of cattle exported to each market varies each year (Figure 9).

Table 40. There were very few voyages to SE Europe and to MISC regions. The MISC region also includes a reasonably disparate group of separate countries that may have quite different characteristics and therefore the findings for this region are of relatively little inferential value.

Figure 14 shows mean mortality rate by destination region.

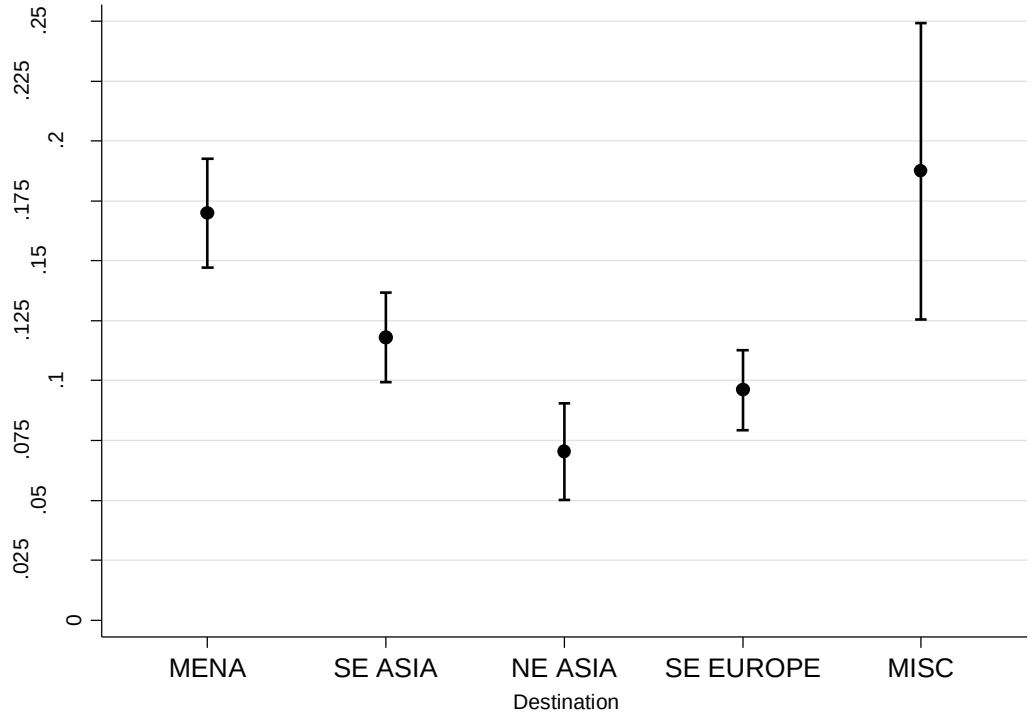


Figure 14: Marginal mean mortality rate by destination region (deaths per 1,000 cattle-days at risk). Bars represent 95% confidence intervals.

The primary interest is in the three regions that have accounted for most voyages: MENA, SE Asia and NE Asia. Within these three regions, the overall mortality rate for MENA was significantly higher than both SE Asia ($p<0.001$) and NE Asia ($P<0.001$). The mortality rate for SE Asia was higher than for NE ASIA ($p=0.001$). It is important to note that these comparisons involved appropriate adjustment for voyage duration to account for the fact that voyages to Asian regions are likely to be shorter in duration than voyages to the MENA.

Further analyses were conducted to explore associations between region and year and month of year. These were limited to MENA and SE Asia because the other regions had fewer voyages (particularly SE Europe) or were miscellaneous aggregations of non-similar voyages with no voyages in some months or years (MISC).

Figure 15 shows mean mortality rate by year for voyages to MENA and SE Asia only.

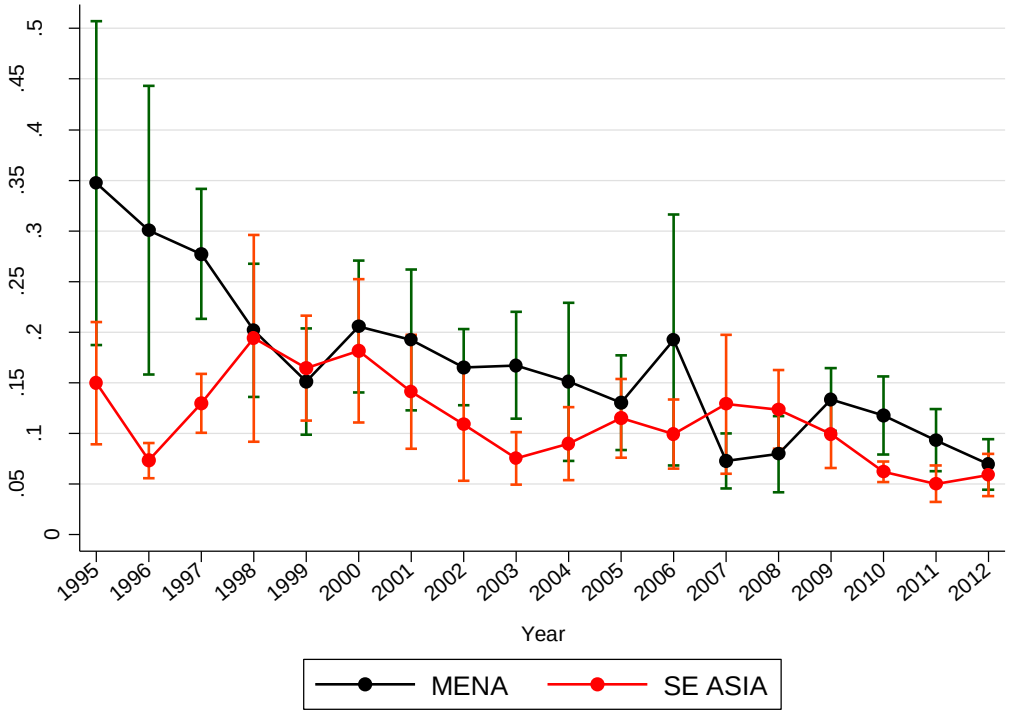


Figure 15: Marginal mean mortality rate by year for voyages to MENA and SE Asia only (deaths per 1,000 cattle-days at risk). Bars represent 95% confidence intervals.

Mortality rates for both MENA and SE Asia showed a progressive decline over time with variation in any particular year. There were noticeable declines for SE Asia from 2000 to 2003 and 2009 onwards, and for MENA from 2006 to 2007 and from 2009 onwards.

The sharp rise for MENA in 2006 was due to a single voyage from Portland/Fremantle to Israel that had a reportable mortality event. When the analysis was repeated with this one voyage filtered, the sharp rise for MENA in 2006 was no longer present. The mortality investigation report for this voyage attributed deaths to pneumonia, heat stress and leg injuries/septicaemia, as the main causes and this mortality event was one of the factors that contributed to the development of this project.

Figure 16 shows mean mortality rate for each destination region arranged by year-group (1995-2002, 2003-2009, 2010-2012). Inspection of Figure 16 supports the conclusion that there has been a progressive reduction in mortality rate over time, though it is more apparent in some regions than in others and there is a suggestion of a rise in mortality rate in NE Asia in the most recent year-group. The wide confidence intervals for MISC reflect the lower number of voyages and the more disparate collection of destinations in this category. Voyages to SE Europe only commenced in the 2010-2012 interval.

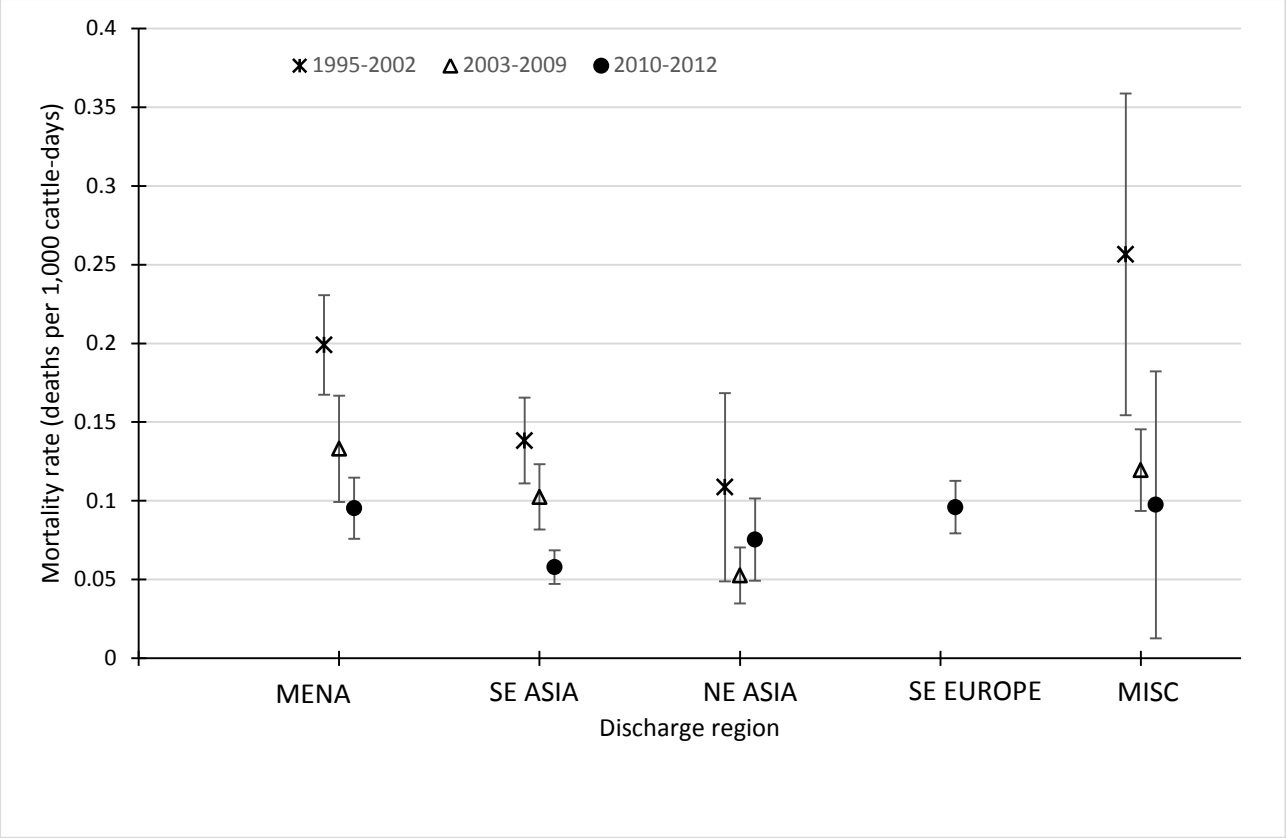


Figure 16: Marginal mean mortality rate for each destination region arranged by year-group (1995-2002, 2003-2009, 2010-2012). Bars represent 95% confidence intervals.

Figure 17 and Figure 18 show the same broad pattern of a general reduction in mortality rate from the oldest time period (1995-2002) to the most recent time period (2010-2012) though in some months this was not always consistent. As an example, Summer mortality rate for voyages to MENA showed relatively little reduction over time (December and January, Figure 17). In contrast, the Summer rise in mortality for voyages to SE Asia is still apparent in the most recent time period (2010-2012), however it has been greatly ameliorated in comparison to the 1995-2002 period (Figure 18).

The rise in mortality rate in late Winter for MENA does represent a significant change. The mortality rate in July was significantly higher than April ($p=0.02$) and September ($p=0.04$).

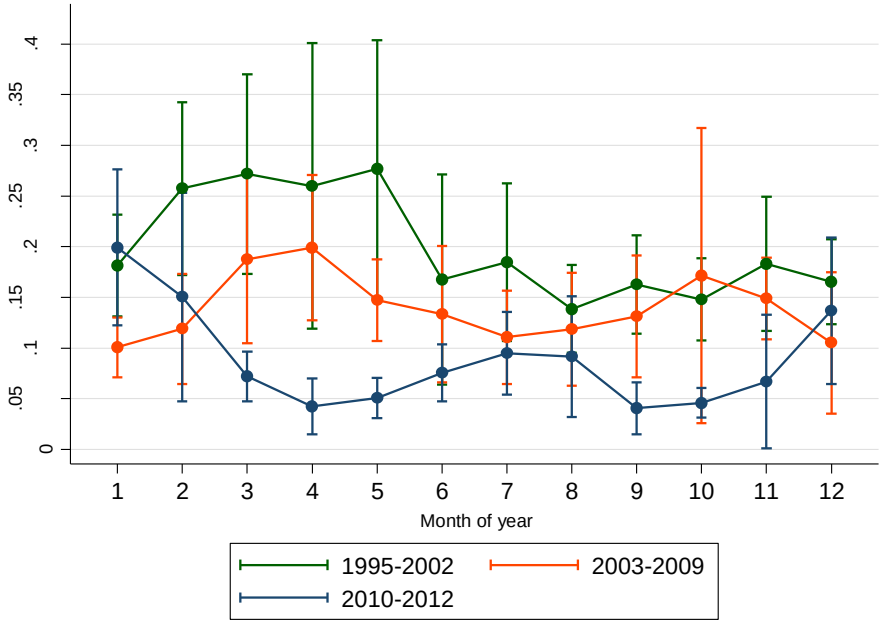


Figure 17: Mean mortality rate by month of year and year group for voyages to MENA only. Bars represent 95% confidence intervals.

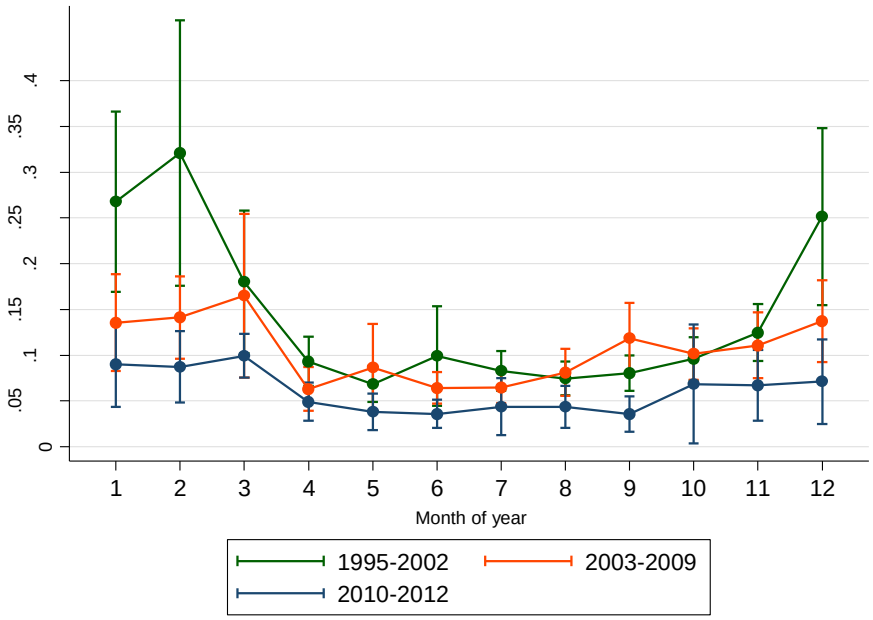


Figure 18: Mean mortality rate by month of year and year group for voyages to SE Asia only. Bars represent 95% confidence intervals.

9.6 Port of loading

The database recorded the port of loading for all voyages. Ports were classified into a three level categorical variable as follows:

- North-west: all ports in Western Australia north of Geraldton and including Dampier, Port Hedland, Broome, Wyndham and Darwin;
- South: all ports from Geraldton south and around to South Australia and Tasmania and including Geraldton, Fremantle, Bunbury, Esperance, Portland, Adelaide, Geelong and Devonport; and
- Other: all ports in Queensland and New South Wales and including Mourilyan, Weipa, Karumba, Townsville, Mackay, Gladstone, Brisbane, Port Kembla, Newcastle, Sydney and any ports that were not able to be identified.

Figure 19 shows mortality rate by load port region within Australia and destination region, for voyages to MENA and SE Asia only.

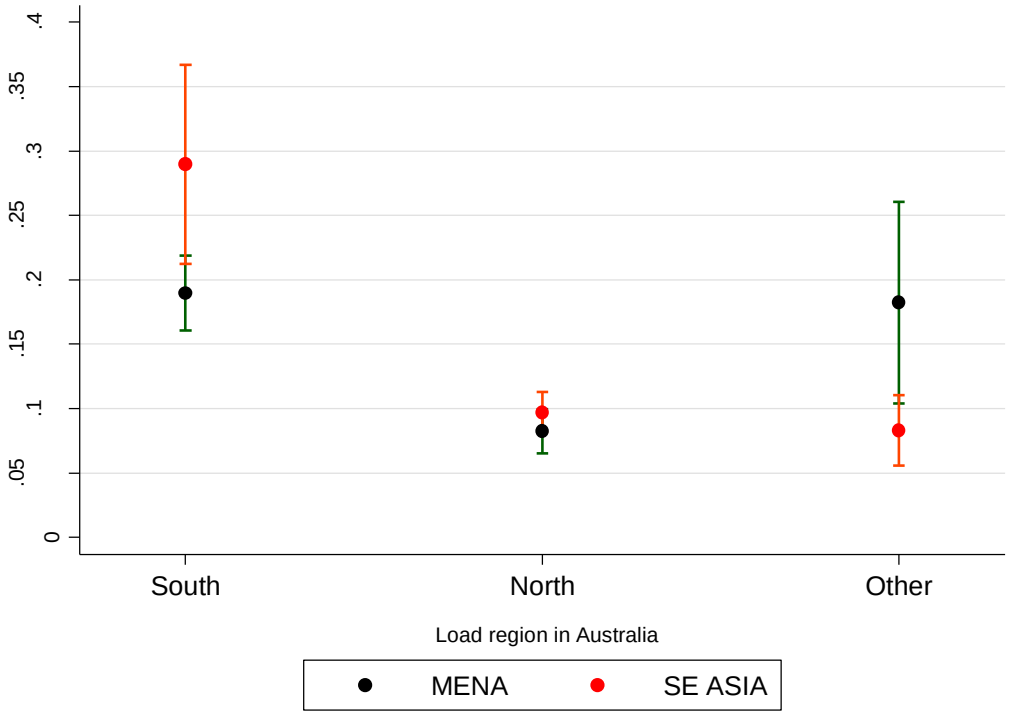


Figure 19: Mean mortality rate by load port region within Australia and destination region, for voyages to MENA and SE Asia only (1995-2012). Bars represent 95% confidence intervals. Data derived from all years combined.

Mortality rates for cattle loaded in the north and going to MENA or SE Asia were significantly lower than mortality rates for cattle loaded in the south and going to the same destination region ($p<0.001$).

For cattle loaded in the south, the mortality rate for voyages to SE Asia was significantly higher than the mortality rate for voyages going to MENA ($p=0.008$). There was no

difference in mortality rate by destination (MENA vs SE Asia) for cattle loaded in the north (p=0.2).

The finding that adjusted mortality rates (deaths per 1,000 cattle-days) were higher for southern cattle going to SE Asia than for southern cattle going to MENA was unexpected. Further analyses were conducted, using data subsets representing the two more recent time periods.

Figure 20 shows mean mortality rate by load port region within Australia and destination region, for voyages to MENA and SE Asia only and where the data were limited to the period from 2003-2012 only. This was done to use more recent data to explore possible explanations for higher mortality risk in voyages travelling to SE Asia.

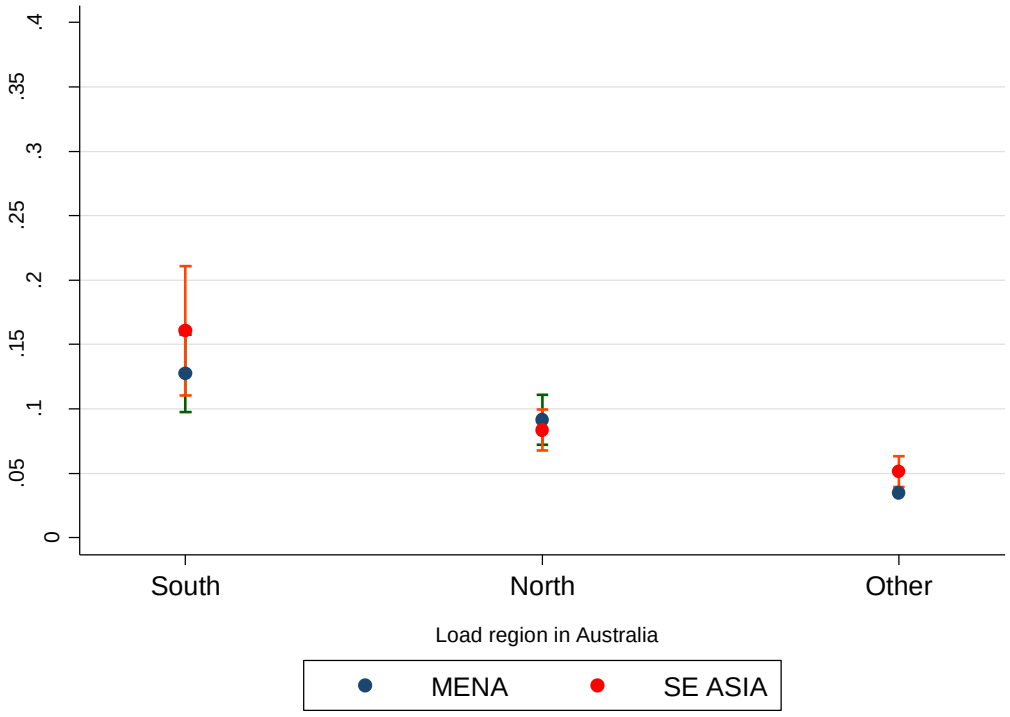


Figure 20: Mean mortality rate by load port region within Australia and destination region, for voyages to MENA and SE Asia only. Bars represent 95% confidence intervals. Data restricted to 2003-2012.

When the effects of load port and destination were assessed using only the data from 2003-2012, there was no difference in mortality rate for cattle loaded in southern ports and going to MENA vs those loaded in southern ports and going to SE Asia (p=0.3). There was also no difference for cattle loaded in northern ports and going to MENA compared to SE Asia (p=0.6).

For voyages to MENA, there was a tendency for southern loaded cattle to have a higher mortality rate than northern loaded cattle (p=0.053). For voyages to SE Asia, southern loaded cattle had a higher mortality rate than northern loaded cattle (p<0.001).

Further comparisons were then performed on the mean mortality estimates by load port and destination region for 2010-2012 (Figure 21).

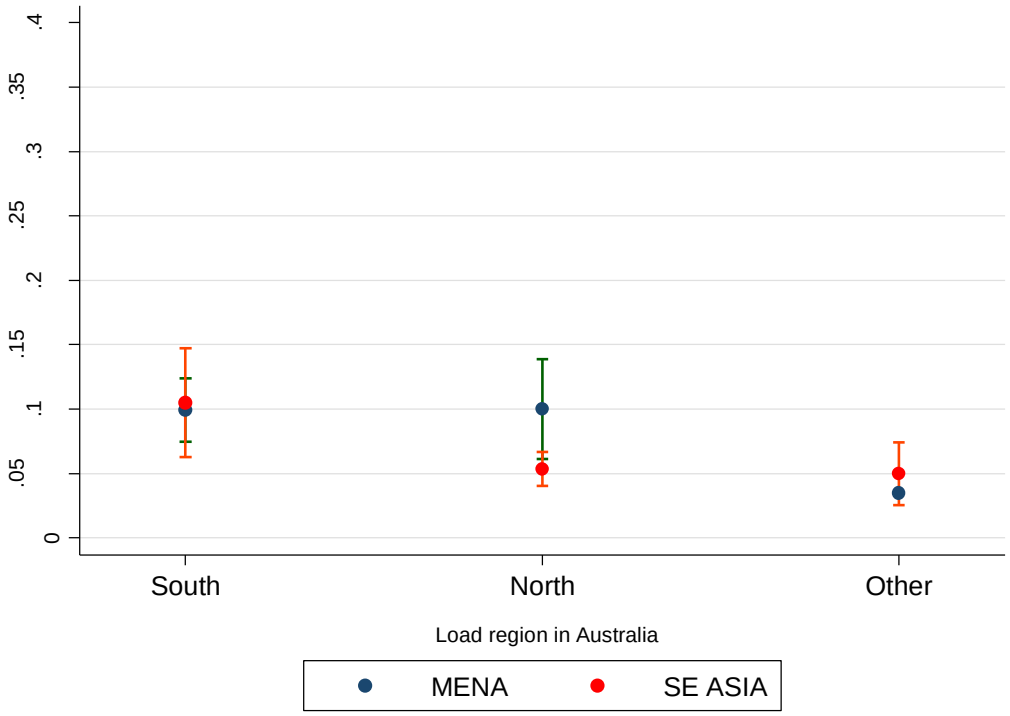


Figure 21: Mean mortality rate by load port region within Australia and destination region, for voyages to MENA and SE Asia only. Bars represent 95% confidence intervals. Data restricted to 2010-2012.

There was no difference in mortality rate for cattle going to MENA if they were loaded in northern vs southern ports (p=0.9).

For cattle going to SE Asia, the mortality rate for cattle loaded in the south was higher than the mortality rate for cattle loaded in the north (p=0.006).

There was no difference in mortality rate for cattle loaded in southern ports and heading to MENA vs SE Asia (p=0.8).

For cattle loaded in the north, voyages travelling to MENA had a higher mortality rate than voyages going to SE Asia (p=0.01).

This was explored further by fitting a model that included season (Summer, Autumn, Winter, Spring; Figures 22 and 23).

Figure 22 shows mean mortality rate by load port region within Australia (South vs North) and season of departure, for voyages to MENA only and limited to 2010-2012. Figure 23 shows similar data, but for voyages to SE Asia only and limited to 2010-2012.

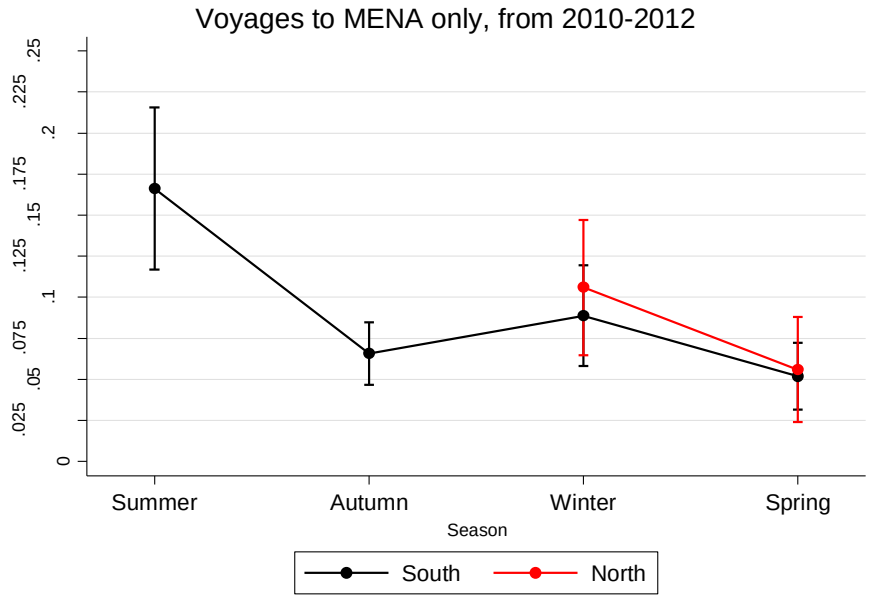


Figure 22: Mean mortality rate by load port region within Australia (South vs North) and season of departure, for voyages to MENA only. Bars represent 95% confidence intervals. Data restricted to 2010-2012.

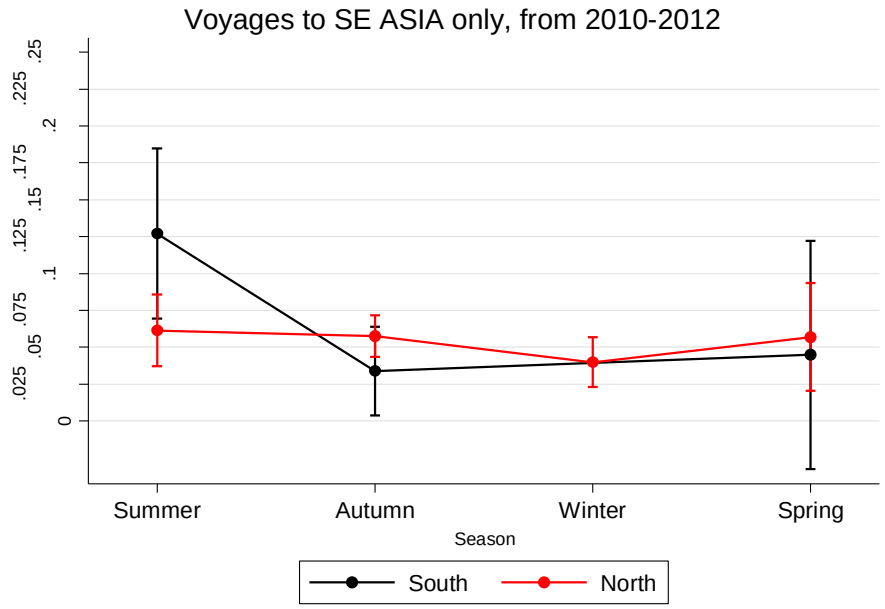


Figure 23: Mean mortality rate by load port region within Australia (South vs North) and season of departure, for voyages to SE Asia only. Bars represent 95% confidence intervals. Data restricted to 2010-2012.

The most interesting finding is seen in Figure 23 for SE Asia voyages where voyages were recorded in all seasons from both north and south ports of loading. The elevated mortality risk in Summer appears to be limited to cattle loaded in southern ports, whereas there is little seasonal change in mortality risk for those cattle loaded in northern ports. It is not possible to

explore these associations for voyages to the Middle East and North Africa, because most voyages to these destinations were loaded in southern ports. Voyages from northern ports were only recorded during Winter and Spring.

The same general pattern was seen for southern loaded cattle in both destination groups, in that Summer voyages had a higher mortality rate than voyages in other seasons.

In summary, the findings indicate that there has been a progressive reduction over time in mortality rate, best viewed by looking at long term plots of mortality rate by year from 1995 to 2012.

More detailed assessment is limited to the most recent time period (2010-2012) since this is likely to best reflect current performance and practices.

Monthly patterns indicate a rise in mortality rate during Summer months and a decline in Winter months. The Summer rise is most prominent for voyages to MENA and much less prominent for voyages to SE Asia. There was a small but significant Winter rise in mortality for voyages to MENA that was not apparent for voyages to SE Asia.

There was an effect of port of loading which may be a proxy for cattle species. Our ability to explore this effect was limited because voyages to MENA did not have any cattle loaded in the north during Summer and Autumn, and because we were not able to collect detailed information on species of cattle in this project. Our results do indicate that for SE Asian voyages, the Summer peak in mortality appears to be driven by cattle loaded in the south and there was no real change in mortality rate through the year for cattle loaded in the north. Southern loaded cattle show the same Summer peak for voyages to MENA.

Other sources have reported that cattle loaded from northern ports are more likely to be *Bos indicus* genotype than *Bos taurus*. [68] Cattle loaded in the north are also considered more likely to have been raised in the north and may have more exposure and adaptation to northern climatic extremes including higher temperatures and humidity. Cattle loaded in the south may be more difficult to characterise. Southern cattle may be mathematically more likely to include temperate genotypes such as *Bos taurus* than tropically adapted genotypes such as *Bos indicus,* however we would expect there to be more diversity in the south and also it is considered more likely that some cattle loaded in the south may have originated from northern properties, whereas it may be less likely for cattle loaded in the north to have originated from southern properties. It would be of value to the industry to have breed (and other relevant potential risk factors) recorded as a routine in industry datasets to facilitate better use of statistical analyses to understand risk and inform risk management.

Norris et al [69] have reported that *Bos indicus* cattle had lower mortality percentages than *Bos taurus* cattle in their study.

It seems plausible that the elevated mortality rate in our study for cattle loaded in the south during Summer may be due in part to genotype, as well as other factors such as seasonal voyage conditions.

[68] DAFWA (2009)
[69] Norris et al. (2003)

It is our understanding that voyages to NE Asia and SE Europe may be more likely to include breeder cattle than voyages to MENA and SE Asia. Anecdotal information suggests that breeder cattle may be more valuable than feeder/slaughter cattle and may be managed differently during a voyage (for example lower stocking density and different bedding and perhaps different preparations including pre-export treatments and vaccinations). We did not have detailed data on animal type (breeder vs slaughter or feeder animal) and we had relatively less data on voyages to SE Europe and NE Asia, so we were not able to characterise mortality rates for these destination areas with the same detail. Further work is needed to describe mortality rate patterns for these areas and to understand drivers.

There appears to be some level of uncertainty over the possible role of season in mortality risk in land-based feedlot cattle. Seasonal variation in mortality in feedlot cattle is well described with peak mortality occurring in Autumn and Winter in some studies [70] and in Spring-Summer in others. [71] A recent survey of Australian feedlots reported that the seasonal pattern of mortality risk over the course of a 12-month period appeared to be related more to incoming numbers of cattle (density) and time post feedlot induction and less related to seasonal effects per se. [72]

The combined effects of northern monsoon rainfall that limit the ability of northern producers to muster and transport cattle during the wet, summer months, and seasonal constraints for southern cattle due to ASEL restricting exports of *Bos taurus* cattle between May and October, are likely to contribute to an increased number of *Bos taurus* cattle being exported from southern ports between November and April.

Figure 24 shows monthly total cattle numbers loaded for export from northern or southern ports, for voyages to SE Asia only and for the period 2010-2012. Figure 24 is consistent with the hypothesised description of factors modifying numbers of cattle exported. Relatively more cattle were loaded from southern ports in January and February, than in all other months, and relatively fewer cattle were loaded from northern ports in these months.

[70] Loneragan et al. (2001); Ribble et al. (1995)
[71] Babcock et al. (2013)
[72] Perkins (2013)

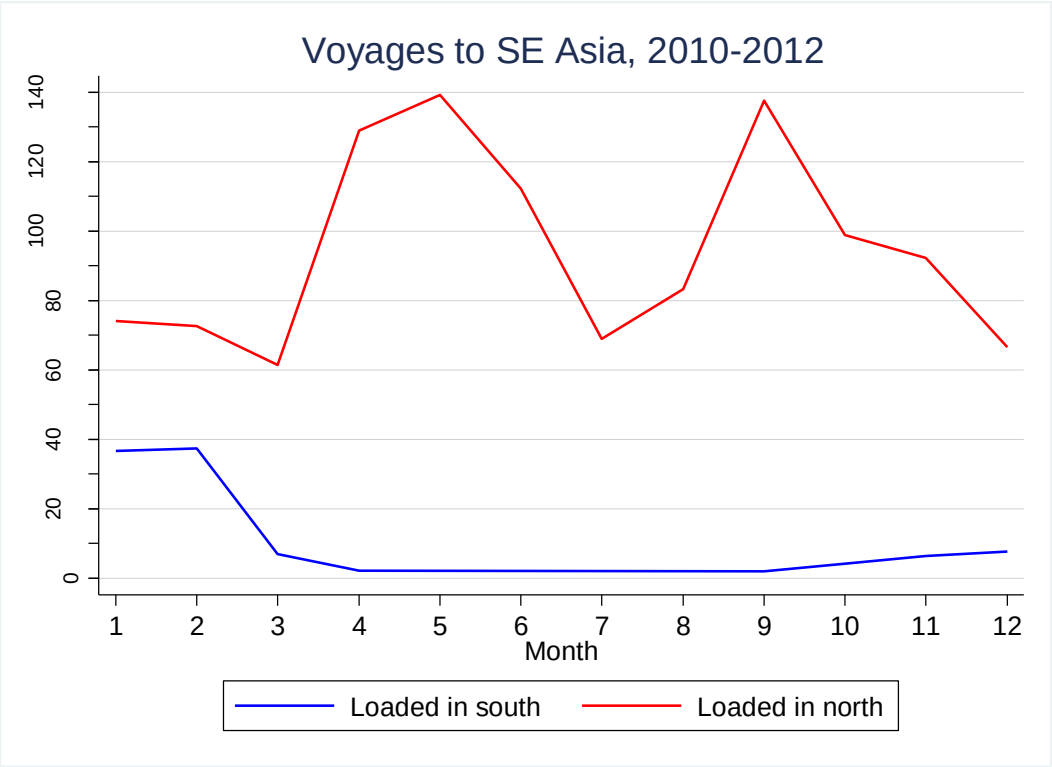


Figure 24: Plot of monthly total cattle numbers loaded for export from northern or southern ports, for voyages to SE Asia only and for the period 2010-2012.

Figure 25 shows monthly total cattle numbers loaded for export from northern or southern ports, for voyages to MENA only and for the period 2010-2012.

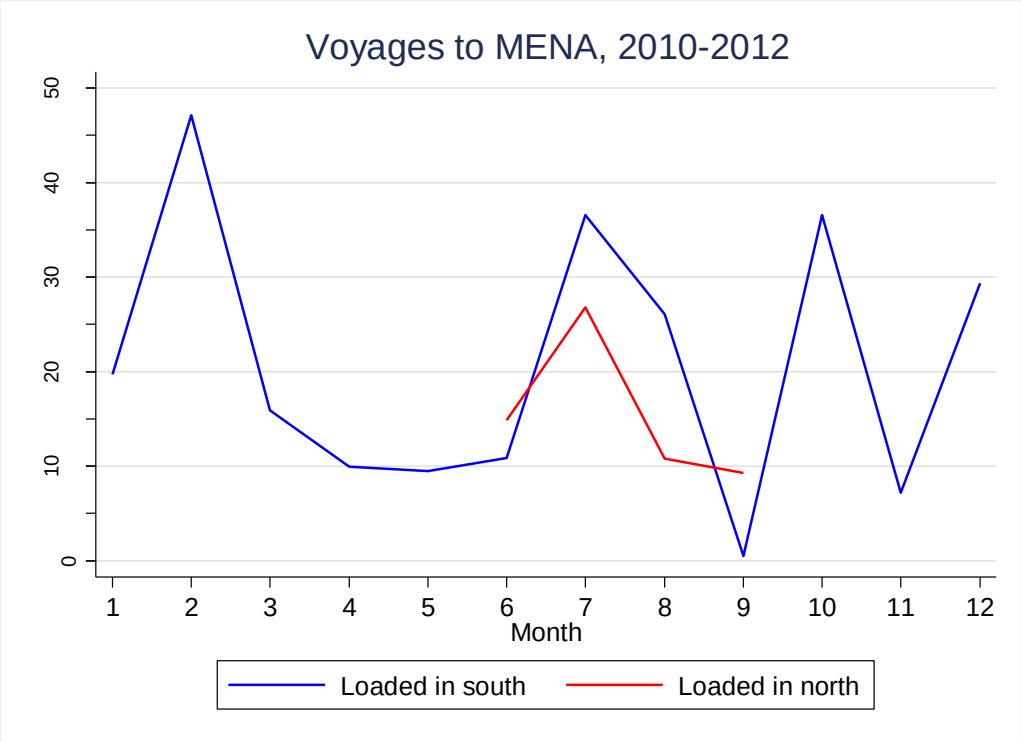


Figure 25: Plot of monthly total cattle numbers loaded for export from northern or southern ports, for voyages to MENA only and for the period 2010-2012.

The pattern for southern ports is consistent with a large increase in cattle exported in Summer and this increase may be sufficient for disease risk to be increased due to animal density and co-mingling and other related factors that are independent of season per se. These findings stimulate questions about whether the apparent seasonal pattern in mortality rate may be explained in part by factors associated with animal density (stress, co-mingling, exposure risk, etc) rather than direct season effects associated with changes in environmental or climatic factors. These same issues are topical in understanding seasonal patterns in land-based feedlot mortality risk and there is evidence suggesting that seasonal changes in animal density may be exerting a bigger effect on mortality risk than seasonal climatic variation. [73] Further work is needed to explore this issue.

The pattern for northern exports to SE Asia is bimodal with peaks in April-May and September, perhaps coinciding with first and second round musters. It is also apparent that cattle exports from the north continue all year round so the seasonal conditions may impede, however they do not prevent cattle aggregation and transport during the wet season.

It is more difficult to explain the pattern for exports to MENA. Cattle loaded in southern ports appear to peak at February, July and October. It is not clear what may be influencing the variation in numbers. There are too few data points for northern cattle to the MENA for a pattern to be appreciated. The results appear to support a hypothesis that MENA markets

[73] Ibid.

may prefer cattle from southern ports, perhaps because of a preference for *Bos taurus* genotypes.

9.7 Daily voyage mortality

Data on daily mortality for voyages were derived from the SMDB for twenty voyages participating in the project. These included 19 voyages that had contributed mortality data and samples for Stage 2 or 3 of the project and one additional voyage (voyage #18) where cattle had been sampled during the assembly period (see Section 8) but had not been enrolled for voyage sampling.

SMDB data contained details on counts of cattle deaths by day of voyage. There was one discrepancy in this dataset that was not able to be resolved for this analysis. For voyage #11, the SMDB records included 106 deaths recorded by day of voyage. As indicated in Section 7.1, Australian Government sources had recorded 107 deaths for this voyage. Because there was no alternative data source that allowed identification of the voyage day when the additional death occurred, it was not possible to correct the SMDB data for this purpose. All other details are the same as reported for voyages in earlier sections of this report.

The SMDB dataset was used to produce an analytical dataset made up of one row per animal for each of the 20 voyages. Each row included the outcome for that animal (dead or alive at the end of the voyage) and the time at risk for that animal. For animals that died during the voyage, the time at risk was the days from the start of the voyage until the animal died. For animals that survived the voyage the time at risk was equal to the duration of the voyage in days. This dataset was then analysed using survival analysis techniques.

Table 41 shows summary statistics for the 20 voyages used in survival analyses assessing voyage mortality rate.

Table 41: Summary statistics for 20 voyages used in survival analyses assessing voyage mortality risk.

VoyageID	Month of loading	Total cattle loaded	Total deaths	Voyage mortality	Total voyage length	First discharge	Destination	Split discharge	Split load
	n	n	n	%	d	d			
1	3	9,430	16	0.17	22	17	Red Sea	Yes	No
2	5	9,213	13	0.141	23	22	Mediterranean Sea	No	No
3	8	5,090	4	0.079	19	18	Red Sea	No	No
4	10	10,428	11	0.105	19	15	Red Sea	No	No
5	1	19,990	148	0.74	24	18	Aegean Sea	Yes	No
6	1	3,994	21	0.526	32	27	Black Sea	No	No
7	1	16,255	25	0.154	35	22	Sea of Marmara	Yes	No
8	3	17,449	52	0.297	35	22	Red Sea	Yes	Yes
9	3	10,237	60	0.586	42	27	Sea of Marmara	Yes	No
10	5	12,763	54	0.423	36	30	Black Sea	No	Yes
11	6	9,000	106	1.178	37	36	Sea of Marmara	No	Yes
12	7	9,239	27	0.292	24	22	Red Sea	No	Yes
13	7	1,350	6	0.444	20	18	Persian Gulf	No	Yes
14	8	4,274	15	0.351	35	29	Sea of Marmara	Yes	Yes
15	9	12,256	61	0.498	31	27	Sea of Marmara	No	Yes
16	1	9,811	17	0.173	22	17	Red Sea	No	No
17	4	7,811	39	0.499	34	30	Sea of Marmara	No	Yes
18	4	9,068	33	0.364	34	33	Sea of Marmara	No	Yes
20	5	11,537	37	0.321	37	30	Sea of Marmara	Yes	Yes
21	6	8,209	21	0.256	30	23	Red Sea	No	Yes
Total		197,404	766	0.388					

Table 42 shows the results from a series of univariable survival analyses. Each analysis had the same outcome (count of deaths per animal-time-at-risk) and had one explanatory variable added at a time to explore associations.

Survival analysis is a specific type of statistical analysis most commonly applied when there is a specific outcome event of interest (in this case, death of an animal) and where there is interest in modelling the time to occurrence of the event (survival time). All animals in the dataset are considered to be eligible to experience the event (death) at the start of the study period (beginning of the voyage). For those animals that die, the time to the event is the days from start of the voyage until death. For those animals that survive through to the end of the voyage, we only know that they were alive at the end of the study period, meaning that they did not experience the event during the study period. These animals are said to be censored at the end of the voyage. Survival analyses are typically reported using a measure called the hazard ratio (HR). This is broadly equivalent to a relative risk (RR) estimated from a Poisson statistical model.

In the following table (Table 42), results are provided of univariable screening analyses run using survival analysis. Each variable was assessed in a separate analysis as the only explanatory variable in a survival model with death as the defined event of interest. Each variable was coded as a categorical variable.

The first variable represented destination region. Two separate screening analyses were performed for destination. The first used a two-level (or binary) variable with one level being those voyages going to the Aegean Sea, Sea of Marmara and the Black Sea (all reasonably geographically proximate). The other level included voyages going to the Persian Gulf (only a single voyage), ports in the Red Sea and one voyage that went to a port in the lower Mediterranean Sea.

One level of each variable is selected as a reference level for comparison purposes. When the survival analysis is run, it compares all other levels in that variable to the reference level.

The second analysis with destination used a six-level variable coding for different destination areas in more detail.

Looking at the result in Table 42 for the two-level form of destination, it can be interpreted as follows. The analysis reports a hazard ratio (HR) which is literally a ratio of the hazard (likelihood of death) in one level of the variable, compared to the hazard for the reference level of the variable. In the case of destination, there are only two levels, so there is only one HR reported.

Those voyages going to the Agean and Black Seas had a 1.65-fold higher hazard (Hazard ratio = 1.65) compared to those voyages going to the Red Sea and other related destinations. This means a 1.65 higher likelihood of death occurring per unit time during the voyage for voyages going to the Agean and Black Seas compared to the reference level.

The row for destination provides a range of other statistical measures (standard error for the HR estimate and a 95% confidence interval and a p-value). The p-value is 0.03 (<0.05 and therefore significant) which means that the hazard ratio is significantly different to 1. If there was no difference in hazard between the two destination levels, the HR would be approximately equal to one and the p-value would be >0.05 (not significant).

Notice that for season, the hazard for Summer was used as the reference level. The HR estimates for other seasons were all compared to Summer (the reference level) and the fact that all HR estimates were less than 1, indicated that Autumn, Winter and Spring all had a lower hazard (lower likelihood of death) compared to Summer. However, the p-values were all >0.05, indicating that these comparisons were all non-significant and therefore this screening analysis suggests that there is no significant difference in hazard between any of the seasons.

A more detailed variable coding for destination was also assessed – with multiple levels coding for different ports. The hazards for the Agean Sea and the Persian Gulf appeared to be notably higher than the reference level (Red Sea) and other ports were variable above or below the Red Sea.

There was particular interest in trying to assess whether there might be support for a hypothesis of a higher mortality risk in the part of the voyage after the first destination port.

This is because once the ship has unloaded some cattle at the first port of destination, any further sailing may be at lower speeds and in more protected areas that may be relatively hot and that may be sheltered and exposed to lower wind flows. Each voyage period was divided into the portion before the first destination port and the portion after the first destination port and these two periods were compared to see if there was any evidence for an elevation in hazard (likelihood of death) in the portion of the voyage after the first destination port. The findings suggested that there was no difference in mortality hazard (p=0.4).

There was also no difference in hazard for voyages that involved split loading vs those voyages that did not involve split loading (p=0.9) and there was no difference in hazard for those voyages that had split discharges compared to voyages that did not have split discharges (discharged at more than one port; 0=0.9).

Table 42: Results from univariable survival analyses using proportional hazards cox regression adjusted for clustering at the voyage level. HR=hazard ratio, se=standard error, z=z-statistic, p=p-value, CI=confidence interval for the hazard ratio.

						95% CI	
Variable	**Level**	**HR**	**se**	**z**	**p**	**Upper**	**Lower**
Destination	Red Sea, Persian Gulf, Mediterranean Sea	reference					
	Aegean & Black Seas	1.65	0.37	2.21	0.03	1.06	2.27
Destination	Red Sea	reference			<0.001		
	Aegean Sea	3.51	0.23	18.86	<0.001	3.08	4.00
	Mediterranean Sea	0.72	0.05	-4.76	<0.001	0.63	0.82
	Persian Gulf	3.05	0.26	13.25	<0.001	2.59	3.60
	Russia	1.28	0.17	1.85	0.064	0.99	1.65
	Sea of Marmara	1.33	0.30	1.29	0.20	0.86	2.06
Season of loading	Summer	reference			0.89		
	Autumn	0.75	0.38	-0.56	0.6	0.28	2.04
	Winter	0.88	0.49	-0.23	0.8	0.29	2.64
	Spring	0.75	0.41	-0.53	0.6	0.26	2.16
Discharge period	Before start of first discharge	reference					
(time-varying)	After start of first discharge	0.71	0.29	0.82	0.4	0.32	1.6
Split load	No	reference					
	Yes	0.99	0.37	0.03	0.9	0.48	2.05
Split discharge	No	reference					
	Yes	0.95	0.34	0.14	0.9	0.47	1.92

Screening models are typically run as a form of exploratory analysis, before then running a multivariable statistical model. Multivariable models have one outcome of interest (in this case death/survival as the outcome event) and then have the capacity to include multiple

explanatory variables. The effect of any one explanatory variable in the model is then adjusted for the effects of all other explanatory variables in the model.

An attempt was made to build a multivariable model using variables from the univariable screening. All variables were considered for inclusion and were removed if they were not significant in a backward, stepwise model building approach. The model reduced down to a single effect, coding for destination as a binary variable. The findings were identical to those reported for the univariable screening.

A flexible parametric survival model (Royston-Parmar model) was then applied using the ***stpm2*** package in Stata in an attempt to explore the pattern of daily mortality rate over voyage duration. An initial model was fitted that incorporated proportional hazards assumptions (no interaction between destination and time). The approach involved fitting splines using five degrees of freedom and then generating predicted hazards for defined time periods (based on the range of time periods covered by both levels of the destination variable (out to day 35) and then plotting the estimated mortality rates. A detailed description of the methodology is not provided. This is an application of a relatively complex analysis in order to produce valid daily estimates of mortality rate (deaths per 1,000 cattle-days) for the duration of the voyage.

It is important to understand how these estimates differ from those described in preceding sections of this report.

In section 9.1 the overall mortality rate for 6,443 voyages across the period from 1995-2012 was reported to be 0.13 deaths per 1,000 cattle-days at sea. This was derived from a dataset with one row per voyage that provided data for each voyage on the count of total cattle loaded, the voyage duration in days and the number of deaths during the voyage. Multiplying the number of cattle loaded by the voyage duration in days provided an estimate of cattle days at risk for each voyage. There were a total of 19,765 deaths from a total of 150,459,089.5 cattle-days at sea.

Average voyage mortality rate = $\left(\frac{19{,}765}{150{,}459{,}089.5}\right) * 1{,}000 = 0.13$ deaths per 1,000 cattle-days.

This estimate assumes that mortality rate is constant for every unit of time (every day) throughout the voyage (period of risk). If a ship was loaded with 10,000 cattle, then this estimate is saying that there would be on average 1.3 deaths every day of the voyage.

It is biologically unlikely that the mortality rate during a voyage would be constant throughout the voyage. Some days there will be no deaths. Other days there may be several deaths. If there are risk factors that develop over time (accumulation of waste in pens over time, deterioration of air quality, stress, exposure to pathogens, injury, etc) then mortality risk may be likely to rise over time. If there is a dramatic change in conditions that influences mortality risk (rough weather, breakdown in ventilation systems), then there may be a sudden and short-term rise in mortality rate. Our knowledge from feedlot studies suggests that animals have a progressive rise in disease and mortality risk over the first 2-3 weeks of the feedlot period and then mortality risk falls. Basically those animals that are most likely to get sick will have experienced disease and will either have recovered or died and then morbidity and mortality risk would drop and remain fairly low. The point of this is that it is reasonable to

expect that mortality rate would be dynamic and may change from day to day during a voyage.

We were very interested in trying to produce separate daily estimates of mortality rate based on statistical models that allowed mortality rate to vary from day to day. In order to do this we needed to obtain detailed data on counts of deaths for each voyage day

The Royston-Parmar survival model is a more complex type of survival analysis that allows mortality rate to vary from day to day. This method was applied to the data to allow estimation of daily mortality rate in order to better understand the dynamic nature of mortality risk during a voyage. Model output was used to generate daily estimates of mortality rate for each voyage day, for each of the two levels of destination.

Figure 26 shows the pattern of daily mortality rate estimates. This is the first time that daily mortality rate estimates have been reported for live export cattle voyages that we are aware of. The results (displayed in Figure 26) are important. There is clear evidence that mortality rate during a voyage is not constant.

Daily mortality rate (deaths per 1,000 cattle-days) at the start of the voyage is very low and progressively rises with each subsequent voyage-day. There is a slight flattening of the mortality rate between days 10 to 20 and then a steeper rise after day 20 to a peak at about day 25-30. Mortality rate then falls towards the end of the voyage.

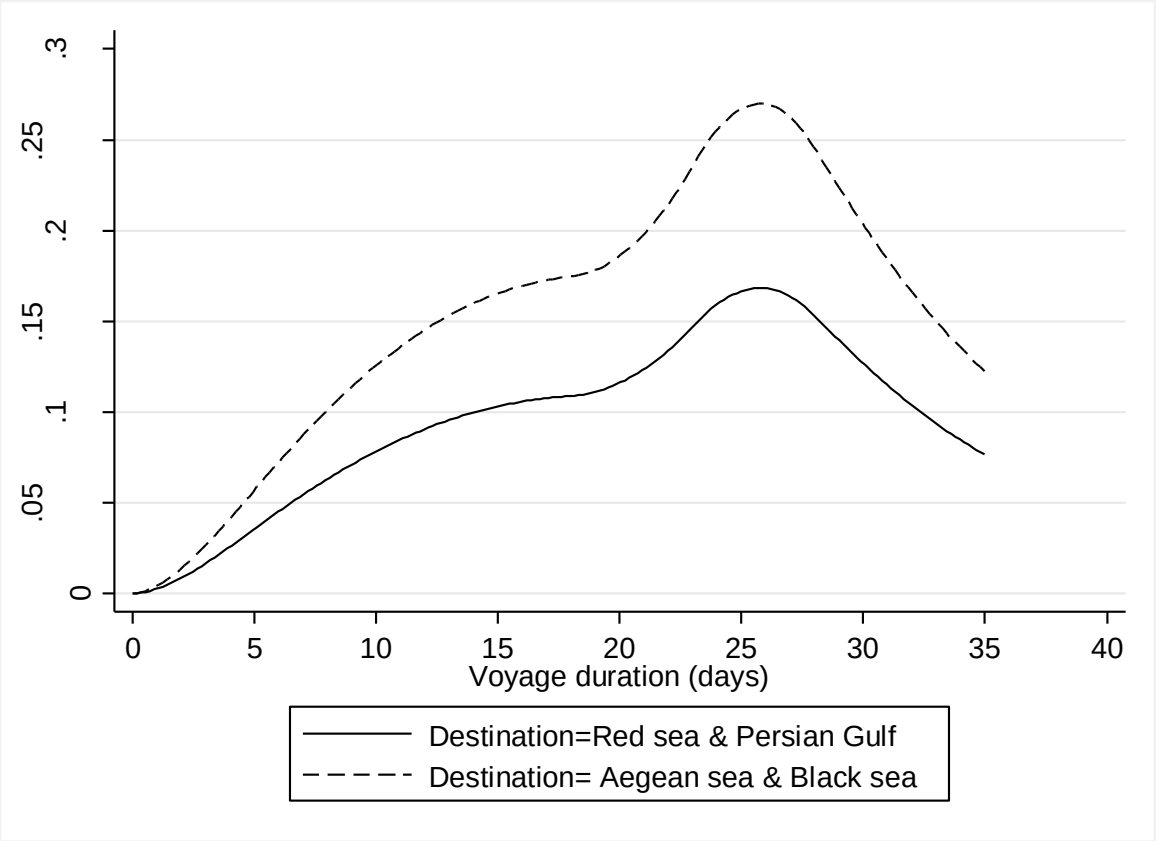


Figure 26: Predicted daily mortality rate (deaths per 1,000 cattle-days) produced from a flexible parametric survival model with an explanatory variable coding for destination region.

Voyage durations are provided in Table 41. It is noted that most voyages to the Red Sea and Persian Gulf were typically shorter than 35 days in duration, and as a result caution should be applied in interpreting estimates of mortality rate around day 35, because they are derived from relatively less data points, particularly for voyages to the Red Sea and the Persian Gulf.

Data was then aggregated from a daily time step to units of one week and a negative binomial regression model run to generate mortality rate estimates for each week of export voyage using all data combined. This is a slightly different modelling approach and at a slightly coarser time-step to the Royston-Parmar model reported above. This model assumes that within a given week, the mortality rate is constant. While this is an over-simplification, it does provide similar output to the Royston-Parmar method.

This approach was done to allow estimation of weekly mortality rates with confidence intervals.

Figure 27 shows mortality rate estimates produced at weekly intervals and clearly shows that mortality rate is low at the beginning of a voyage and then progressively rises to a peak in week four, before declining towards the end of the voyage.

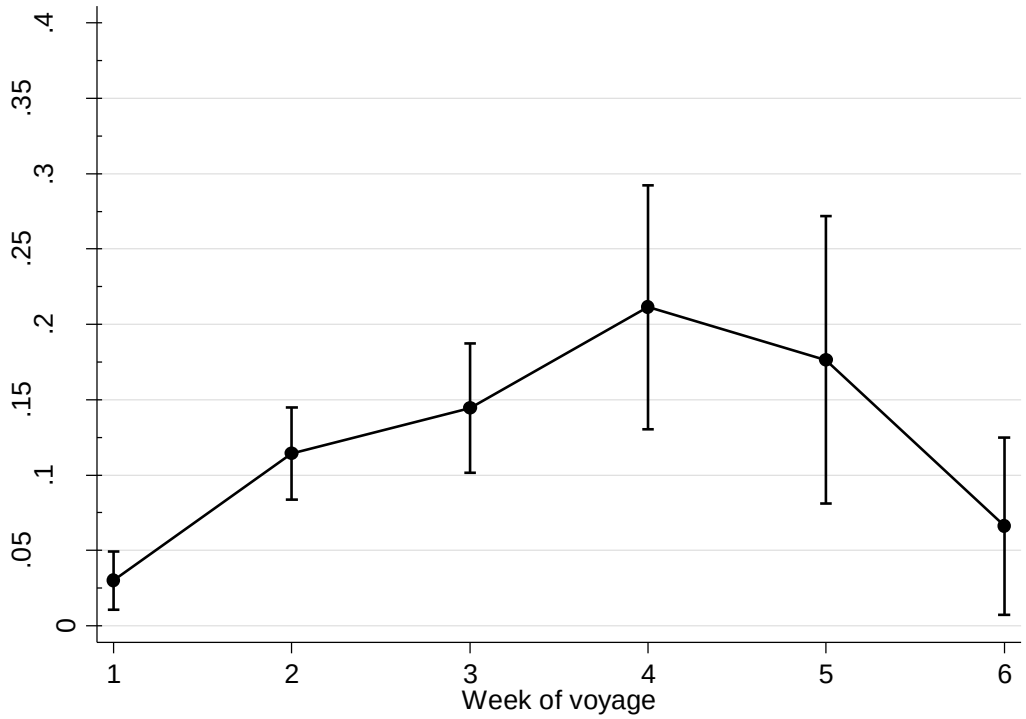


Figure 27: Predicted mean mortality rate (deaths per 1,000 cattle-days) produced for each week of voyage, derived from a negative binomial regression with week fitted as a fixed effect and using data from all 20 voyages. Bars represent 95% confidence interval.

These results are very consistent with our knowledge of mortality rate over time in land-based feedlots.

In land-based feedlots in North America and Canada, peak mortality due to BRD is observed at approximately 2-4 weeks post induction. [74]

An Australian survey of cattle feedlot morbidity and mortality found that peak mortality occurred slightly later, at between 4-6 weeks after feedlot entry (Figure 28). [75]

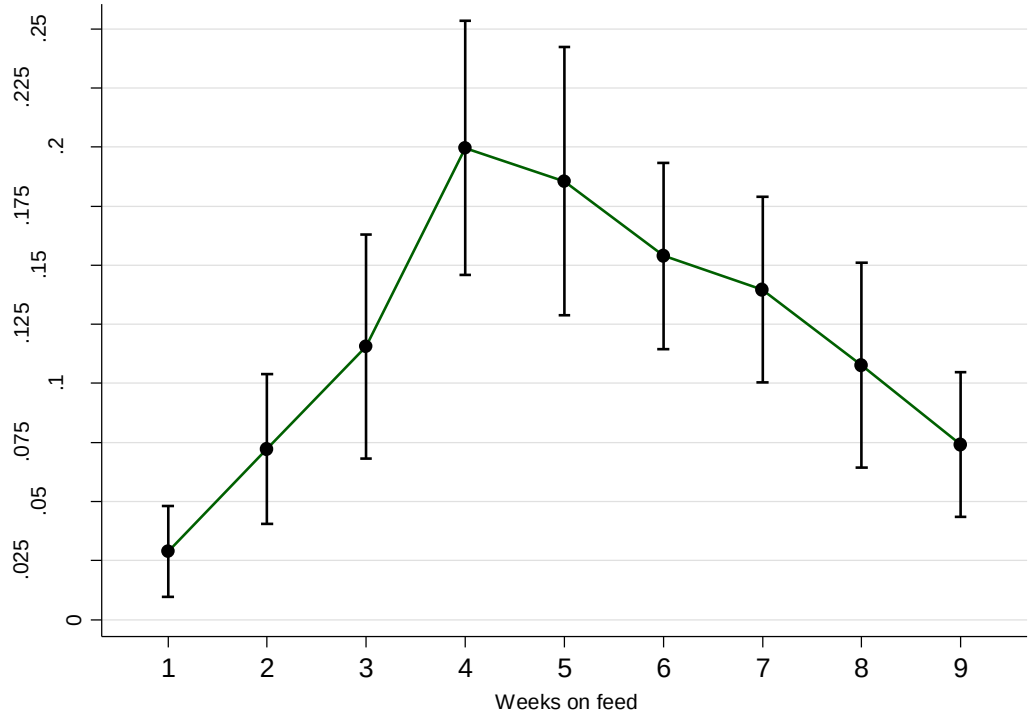


Figure 28: Mortality rate from all causes (deaths per 100 cattle-weeks on feed) reported by weeks on feed for land-based feedlots in Australia. Bars represent 95% confidence interval. [76]

The timing of peak mortality risk is hypothesised to be related to the timing of the main period of exposure of cattle to potential pathogens and associated stressors that may predispose animals to development of disease. The later occurrence of peak mortality in Australian compared to North American cattle, may be because in Australia more cattle are transported directly from property of origin to feedlot without going through saleyards or interim aggregation depots. In contrast, relatively more cattle in North America may experience aggregation or saleyard mixing prior to arrival at the feedlot. This means that exposure and associated mingling stress in North American may occur prior to arrival at the feedlot, whereas in Australia it may occur on arrival at the feedlot.

Under this hypothesis, there is a relatively consistent time window of 4-6 weeks from initial pathogen exposure to peak mortality risk with varied time from induction to peak mortality being explained by variation in when exposure occurs (before or at induction).

[74] Kelly, AP, and Janzen (1986); Ribble et al. (1995); Wilson, Church, and Acres (1985)
[75] Perkins (2013)
[76] Ibid.

Applying this hypothesis to the export industry would mean that we might expect most cattle to be subject to mingling and exposure risk on arrival at the assembly depot, akin to induction at a land-based feedlot. The peak mortality rate for export voyages occurred around week 4 of the voyage, which is comparable to the peak mortality rate from all causes for land-based feedlots in Australia.

We expect that many of the causal factors influencing occurrence of BRD in export cattle will be the same as for BRD in land-based feedlots. Our results indicate that the pathogens involved in BRD in export cattle are the same as those in land-based feedlots. Additional risk factors that may be operating during an export voyage include sea and local weather conditions and the specific deck and pen-level conditions (ventilation, air quality, accumulation and characteristics of bedding and manure, temperature and humidity). There are additional time-based factors on export voyages associated with progressive changes in the pen environments over time (e.g. accumulation of waste products) and changes in the local climate and sea conditions that may be associated in part with the geographic position of the vessel as it crosses the Indian Ocean. For example, hot, humid conditions with little wind in the Intertropical Convergence Zone followed by relatively favourable conditions between the Intertropical Convergence Zone and Gulf of Aden.

The apparently higher mortality rate in cattle destined for the Aegean and Black Sea ports (Turkey, Russian Federation) compared to the Red Sea and Persian Gulf (MENA) is probably due to a combination of the type of cattle on these voyages and seasonal climatic conditions. Six out of the 9 voyages to MENA carried cattle with a high *Bos indicus* content, while voyages to Turkey and the Russian Federation comprised *Bos taurus* feeder and breeder cattle. In addition, 3 out of 11 voyages to Turkey and the Russian Federation sailed from Australia in January, i.e. from the southern hemisphere Summer to the northern hemisphere Winter, and were thus going from one climatic extreme to another. Exposure to temperature extremes without sufficient time for physiologic adaptation may have increased the mortality risk for these voyages, particularly around discharge.

9.8 Cattle movement patterns

Lists of RFID animal-level identification values were obtained for all animals on three study voyages (voyage ID 5, 8 and 12). Records of cattle movements were obtained separately from the Western Australia NLIS database. This allowed descriptive assessment of movement patterns for those cattle exported from ports in Western Australia (some animals on these voyages were loaded from ports in Victoria).

Figure 29 and Figure 30 are intended to demonstrate some of the potential of combining NLIS movement records with additional data linked by unique animal identification numbers (RFID number). The three voyages for which data was available included cattle loaded from Broome and Fremantle within Western Australia. The figures show that cattle originated from across the state.

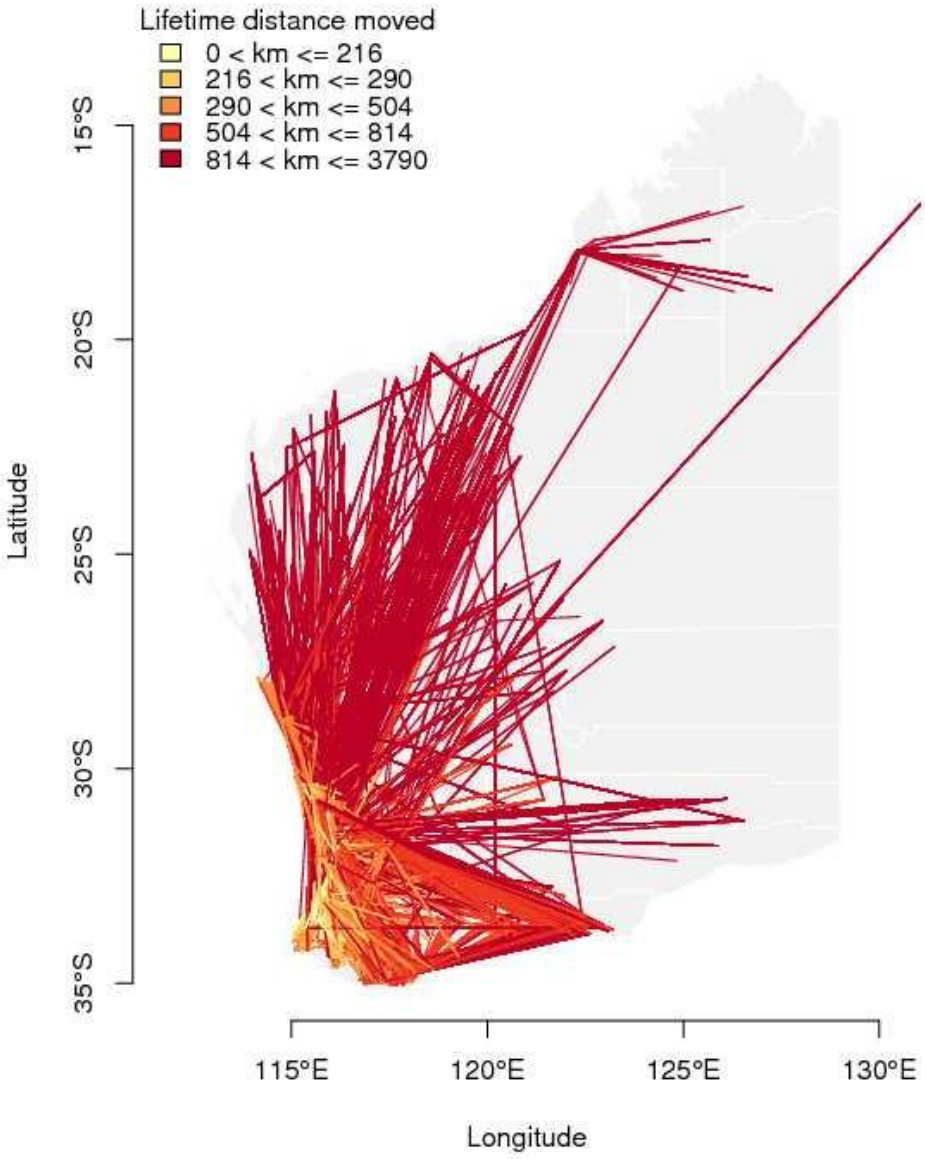


Figure 29: Map of Western Australia showing lines depicting lifetime movement records for cattle exported from WA ports on three export voyages. Colours represent categories of lifetime distance travelled by cattle before export.

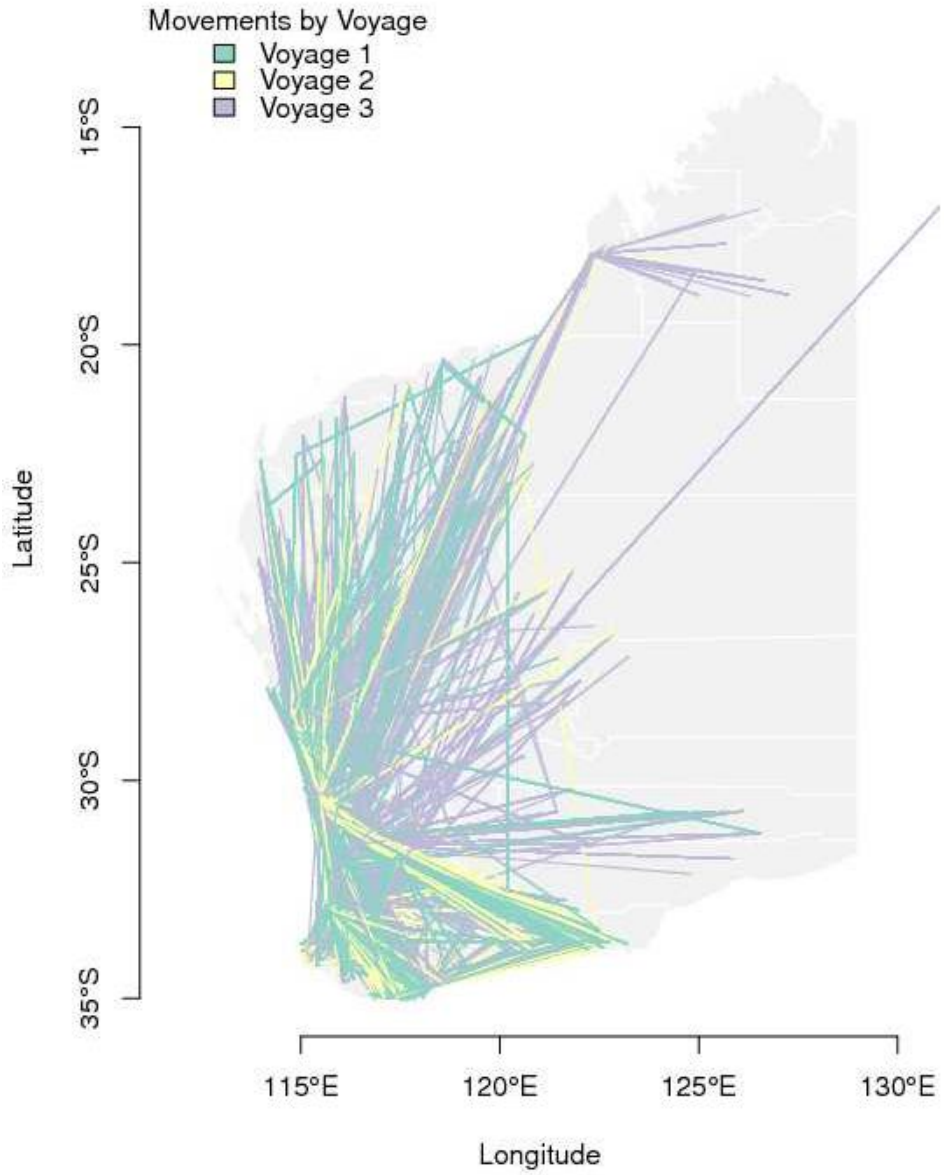


Figure 30: Map of Western Australia showing lines depicting lifetime movement records for cattle exported from WA ports on three export voyages. Colours represent movements for each of the three separate voyages.

Across all cattle movement records, the number of property to property movements prior to export ranged from 1 to 10 for any individual animal. Lifetime movements for individual animals covered distances ranging from 6 to 3,790 kilometres and cattle originated from 105 shires.

Limitations in the data records constrained what we were able to achieve in assessing animal movement patterns. We were only able to obtain individual animal identification lists for three voyages. While these voyages represented many thousands of cattle, the ability to look for large scale patterns in animal movement would be improved if data were able to be obtained from more voyages.

It was not possible to obtain individual animal identification records for all animals that died on voyages enrolled in this study. The ability to record individual animal identification through

RFID or NLIS identification records has tremendous potential to serve as a foundation for additional records that are of value to provide better decisions for industry operators.

Lifetime data such as breed, sex, property of origin, date of birth, etc could be linked to individual animal identification records.

Pre-export treatments or other measures may also be recorded, such as disease testing, vaccination, application of treatments (antibiotic, anthelmintic, parasiticide, etc), animal movements and measures such as body weight, condition score, etc.

Morbidity and mortality records and cause of death can be recorded against individual animal identification records.

An aggregated dataset could then be analysed to look for associations between any possible measures (sex, breed, treatment, origin, movements, time of year, etc) and complex interactions between these measures and defined outcomes such as mortality due to specific causes or morbidity.

The benefit of this information for exporters is making better decisions about selecting animals and managing animals during preparation for export (transport to assembly depot, treatments or vaccinations, etc) to lower morbidity and mortality risk and improve performance and profitability.

10 Development of a shipboard application

10.1 Background

In the planning stages of this project there was interest in development of systems that would allow capture of animal health data onto a hand-held device or laptop during the voyage. Members of the project team had had earlier experience during LIVE.123, where a customised database application had been developed to allow data entry via a laptop during the voyage. During that project the system functioned by having project team members collecting information on paper based records or in notebooks while inspecting animals or performing post mortems and then entering data and information into the database in the team member's cabin.

After initial consultation with industry stakeholders during the first months of this project a decision was made to develop paper-based data recording forms as the major form of data collection during the project. All existing data and information recording systems used by AAVs and the regulatory reporting systems at that time (daily and end of voyage reports) were based on paper reports. There was little general industry support for a centralised database driven system that might be capable of being managed on a laptop and possibly on hand-held devices.

A decision was also made to explore options for development of a prototype information management system (IMS) that would be trialled during the latter stages of the project – a ***shipboard application*** (shipboard app). The purpose of this objective was to test feasibility and practicality of options for use of hand held devices and design of software. The scope was limited to feasibility testing that would inform a final report section on options for devices

and software applications that might be considered for future development. In part this limitation recognised the fact that full development of a read-to-use product would be risky, because stakeholder acceptance would not be guaranteed and the costs associated with producing a ready-to-use product were considered to be higher than the allocated budget.

10.2 Development approach

The approach used for development of an IMS was based on the following general principles:

- The focus was on a system used by an AAV (or stockperson) during a voyage.
- Based on a three level framework:
    - Secure web-mounted database accessible from any internet-capable device (could receive data directly from remote devices).
    - Able to be run as a stand-alone server on a laptop during a voyage, thinking that an AAV would have a laptop running the application in their cabin. The stand-alone server could wirelessly back up the mobile devices when they are in range.
    - Self-contained, mobile app running on a hand-held device such as an Android or iOS device, including smartphones to allow data capture at the point of observation (pen-side or animal-side).
- User interface must be as simple as possible, intuitive and easy to use including:
    - Incorporation of drop down boxes and look-up tables to minimise the need for typing of information, error checking on data entry where possible and effective table design to ensure information is only entered or captured once.
- All components of the system must be relatively cheap, robust, flexible and able to withstand the rigors of the voyage environment.
    - Android and iOS based systems were preferred, because there are many options for developing apps for these systems, they allow choice of many different hand-held devices and the costs for these are relatively low (2011 prices indicated that devices could be purchased for AUD$200 each).
    - High specification, military type devices provide a more rugged option, but have much higher costs for purchase (AUD $2-5,000) and in many cases for app development.
- The minimum outputs were based on regulatory requirements (daily voyage reports and end of voyage reports), recognising that future systems could require the flexibility to add additional output functions based on future quality assurance reporting requirements and other measures supported by industry.
- The system should be able to collect and store a variety of media or data types including simple data (text, numbers that are entered or selected), scanned ear tag ID values, photos/video and audio recordings of observations, as well as importing data from other devices such as climate log devices.
- Modification of existing off-the-shelf apps or software products was selected for initial development of an app, because this allowed relatively low cost development of apps for testing purposes.
- The project team developed a database table diagram and accompanying notes on work and data flows that was based around our knowledge of routine data flows on-board an export ship and reporting requirements. This was used to inform development of all prototypes.

10.3 Prototype systems

Three different prototype systems were developed and tested. A larger number of available systems were assessed and deemed completely unsuitable, as they relied on real time delivery via internet connections.

10.3.1 EpiCollect system

EpiCollect is a data management system developed at Imperial College, London and funded by the Wellcome Trust. EpiCollect can be accessed at www.epicollect.net. It provides a web and mobile app for the generation of forms and websites that can be used for data collection. The app can be run on any Android or iPhone operating system.

The system is intended to allow users to set up a project, design forms for collecting specified data and then use their own mobile phones to collect and upload data to a website, either hosted by the Imperial College using the Google AppEngine, or created by the user.

The system is free to download and relatively simple to use, although configuration is not user-friendly.

The project team developed a prototype system for collection of voyage animal health data as part of this project. Development was done in house by Dr Ben Madin, using information and instructions available on the EpiCollect website. A dedicated site[77] was created to aggregate data from any registered user as a proof of concept. The system was trialled internally by members of the project team, but very early in the development it became apparent that EpiCollect was not going to meet industry needs.

EpiCollect is a useful and low-cost system, but is really best suited to very simple data collection needs, such as surveys. The EpiCollect framework was too simple to allow efficient design of some of the complexities in the table structures, form design and data entry fields that would be required to ensure data was consistently coded between users. In addition the system appeared to be relatively unstable at times, with system crashes potentially resulting in loss of entered data or inability to enter data on occasion. Attempts to access the underlying codebase in the hope of building on it were unsuccessful.

10.3.2 FormEntry system

FormEntry is a development toolkit intended to allow developers to build their own applications for the iPad, iPhone or iPod. More information is available at: http://www.widgetpress.com/formentry

This system was developed for implementation on an iPod touch device, with the capacity to adapt it to other Apple devices (iPad or iPhone).

A prototype system was developed and provided to project team members and to AAVs for trialling.

The development of this prototype system required specific development coding expertise to develop system using a base framework, representing a higher level of development input compare to the EpiCollect prototype.

Figure 31 shows a screen grab of user interface for representative screens from the FormEntry system.

[77] http://exportcollect.ausvet.com.au/

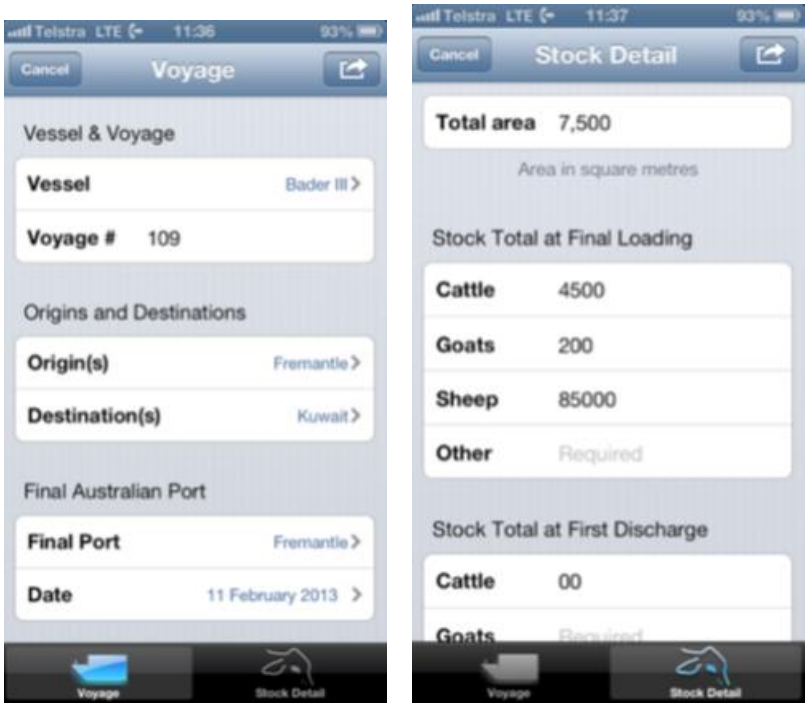


Figure 31: Screen grab of user interface for representative screens from the FormEntry system.

The system was made available for download to selected industry stakeholders and to project team members for trialling. Feedback, both internally and from stakeholders was mixed:

- There was general support for the principle of a system that was based on hand-held devices and that synchronised to a database system.
- There was very clear feedback that the system had to be intuitive and easy to use and the design limitations for FormEntry meant that the prototype system was not able to achieve these objectives.
- The FormEntry approach was consistently more stable and provided more development capacity than EpiCollect.

The FormEntry platform was assessed as having limited capacity to manage the complexity and hierarchical nature of the table structure required for livestock export data and there were problems with importing or synchronising data into the system (necessary to get load plan or animal loading lists into the system at the start of the voyage to act as a denominator for the voyage).

In conclusion the FormEntry option was deemed to be not suitable for widespread use.

At the end of this process we had explored two off-the-shelf options for developing an app for use on export voyages and neither option had allowed development of a system that achieved the desired functions and that was intuitive and easy to use. Our experiences with these off-the-shelf options convinced us that two major design drivers of relatively complexity and hierarchical table structure, and the need for design flexibility to ensure a simple and intuitive user interface, meant that an effective shipboard app would require custom development.

Although neither the EpiCollect nor FormEntry options were satisfactory for the project, the feedback and development issues were valuable steps in defining the restrictions and functional attributes for this app. Many of the principles outlined in the final part of this chapter were established during this phase.

The final step in the prototype development process was to move away from attempting to modify existing off-the-shelf products to a customised product development project.

10.3.3 Custom application

We approached a commercial provider of app and web development services (nowcommsgroup.com and freshweb.com.au) with experience on applications developed for Australian livestock industries, including development of the Stocktake Plus app (stocktakeplus.com.au) for MLA.

A design brief was developed that defined development of:

- A server app running on a linux system that could function as a stand-alone installation on a laptop or as a web-available application if internet services were available.
- An Android app running on hand-held devices for data collection with key functions including:
    - ability to import lists of livestock at load-out based on loading plan for individually identified animals (cattle) or mob-identified animals (sheep).
    - recording of events and conditions during the voyage, including sickness, deaths, sea conditions, and pen/deck/ship conditions.
    - attaching of notes and photos and audio files to any event record.
    - able to synchronise with the server app with two-way data flow.
    - optimized for small screens with fast and simple user interface.
    - support for scanning of animal RFID tags.

This brief included a discussion of the previously identified constraints and requirements created by the need to provide a reliable and easy to use service in a harsh environment.

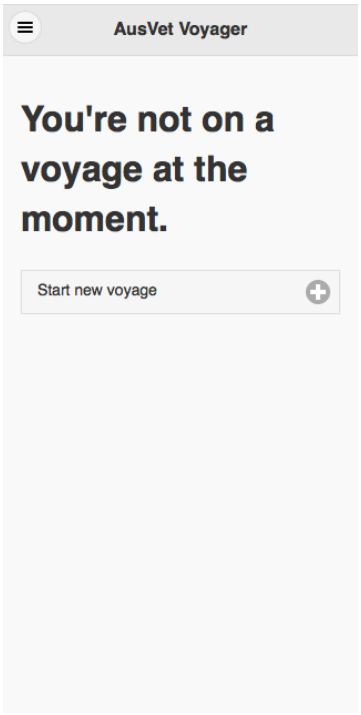
Discussion with the developer indicated that likely costs for completion of a fully functional system with both server and hand-held components would exceed $50,000. This was well above available funding allocated within the existing project budget.

A negotiated approach was then agreed to restrict developer activities to specification of table structure and development of partially functional demonstration modules that showed how specific data entry fields may be managed. Any outputs from this work were intended to inform specification and recommendations for future development of an application for this purpose.

A brief description is provided here of the layout of example screens of the demonstration modules developed in this prototype system.

Figure 32 shows a diagrammatic representation of the opening screen for a custom developed application.

a).



b).

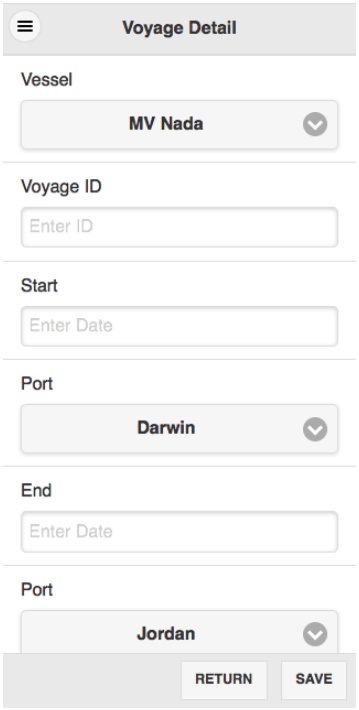


Figure 32: Diagrammatic layout of opening screen shown in a). and screen for entering a new voyage shown in b).

The system is developed specifically for display on small screens and for use on touch-enabled devices.

Options are triggered by pressing symbols with a finger or stylus device. The plus symbol  is used to trigger adding a new record or entry.

A down-arrow symbol  is used to trigger a drop-down list which is displayed in a pop-up dialog box, allowing the user to scroll through options and select an option by pressing it with a finger. The field will then be populated with that option. Figure 33 shows an example drop-down list showing pre-entered options for PORT.

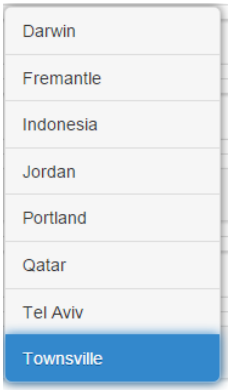


Figure 33: Example drop-down list produced by pressing the arrow beside PORT as displayed in Figure 32 b).

The entries available for selection in a drop down box can be managed through separate screens or a new entry can be recorded by manually typing in a new port name.

A sideways facing symbol  will open a new screen with additional details for that variable.

The small circle symbol containing three horizontal bars  will produce a main menu of available options to choose from, that includes data entry and reporting options. This is an emerging standard for menus on mobile devices.

At any point the user can go straight to a main menu list of major options through the menu  symbol in the upper left of the screen, or can save newly entered data by pressing the save button at the bottom of the screen, or can go back one screen by pressing the return button.

Figure 34 shows example screen-layouts for adding a new vessel to the system.

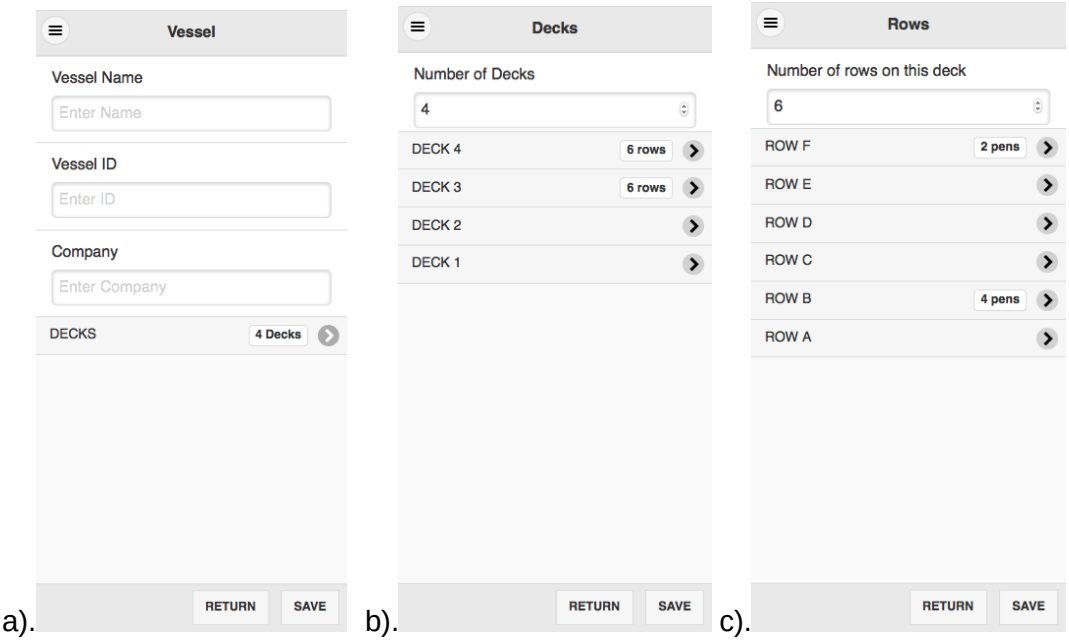
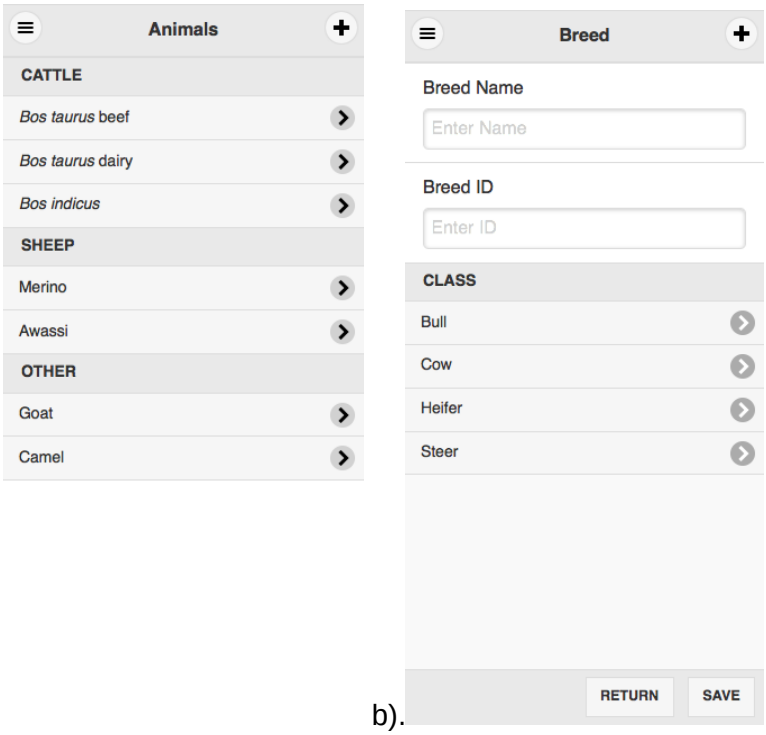


Figure 34: Example screen layouts for adding a new vessel to the system with a). defining the new vessel, b). defining the number of decks and c). defining the rows for each deck.

The system is designed to allow a vessel to be defined with multiple levels of detail down to the number of pens on each deck. The intention is that static ship information would be pre-entered, so that a user chooses the vessel name and then all the details for that vessel are already available for selection and recording.

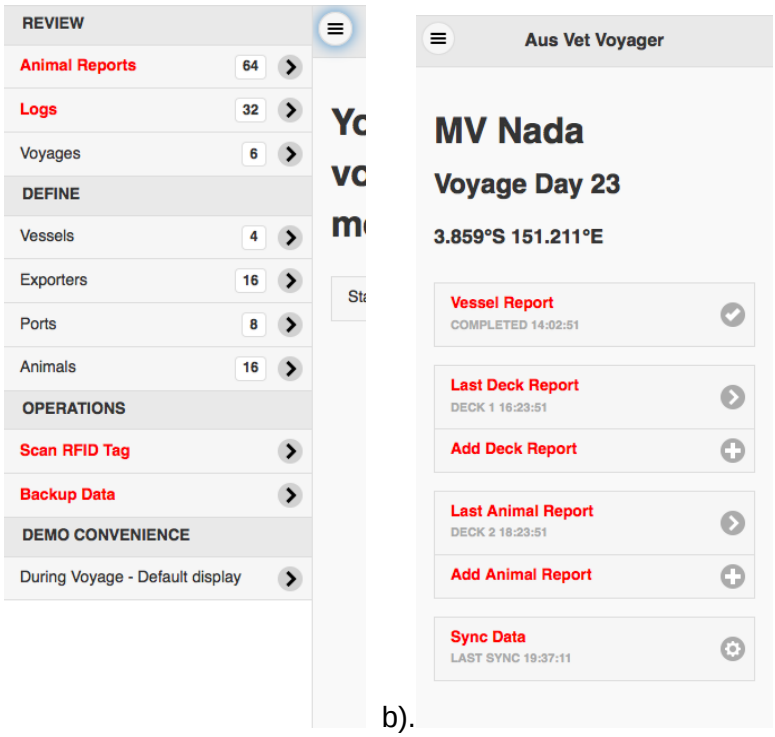
Figure 35 shows example screen-layouts for selecting an animal breed.



a). b).

Figure 35: Example screenshots showing options for selecting animal breed in a). and class in b). and showing the fields where new breeds can be added in b).

Figure 36 shows example screenshots showing a list of summary headings or choice-options (a) and a main user-interface screen during an active voyage where a user may choose between specific options most relevant while a voyage is under way.



a). b).

Figure 36: Example screenshots showing a list of summary headings in a). drawing on data from multiple voyages and b). a main navigation screen active during a voyage where a user may choose between a small number of routine data entry and reporting activities.

From a design perspective the prototype development process reinforced the importance of simplicity, restriction of the amount of information displayed on screen at any point in time, use of simple, intuitive symbols for navigation and key functions, and use of drop down boxes and other aids to limit the amount of new data or information that might have to be manually entered. All of these points contributed to a simple, easy to use interface.

10.4 Database table structure

A simplified database table structure for the IMS is shown below in Figure 37.

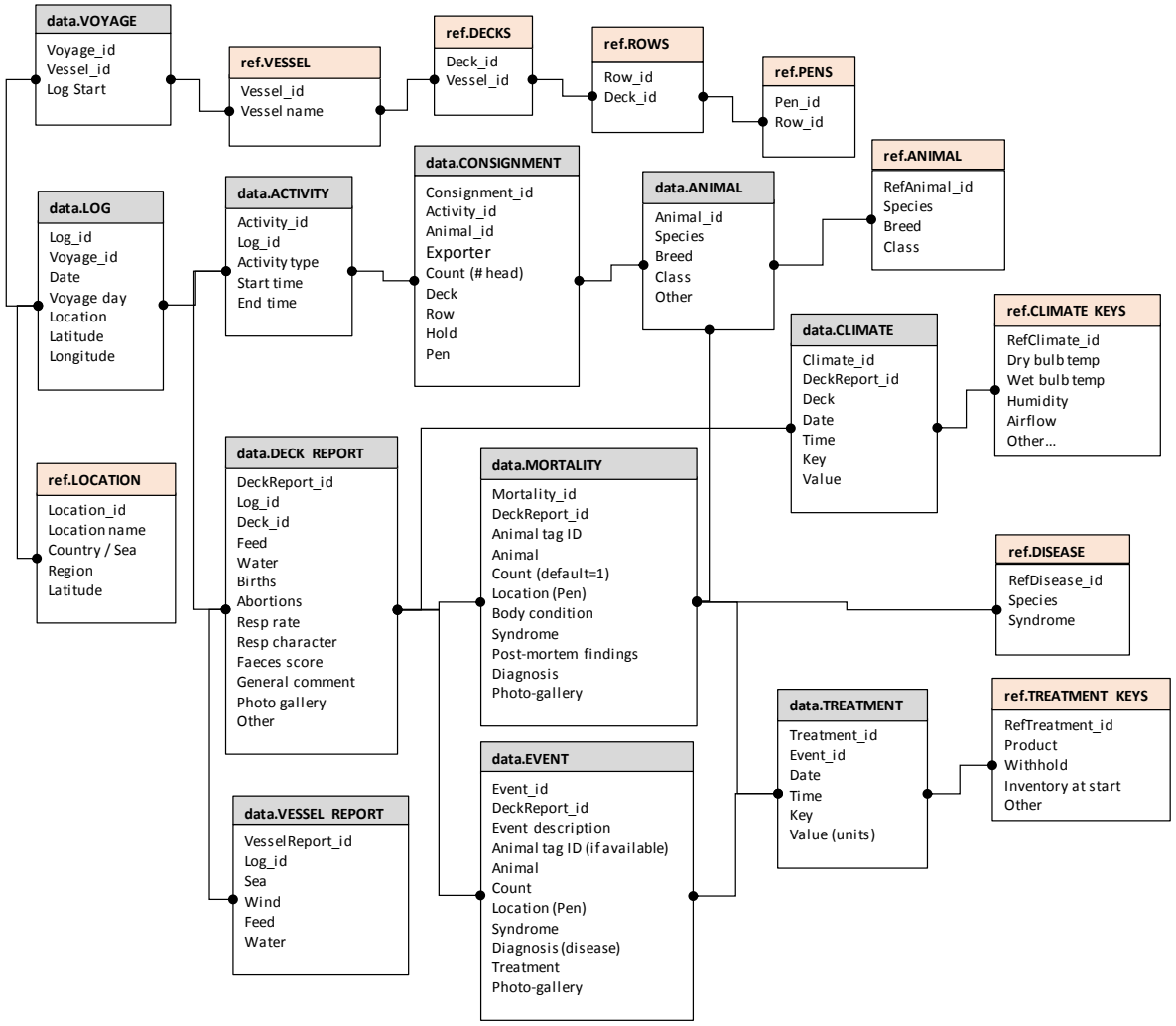


Figure 37: Diagram of table structure for IMS database

Brief explanations are provided here of the format and linkages and different items in the diagram. This is followed by a general description of how the database might function.

Entities are identified by the table headings (bold, shaded first row of each table) and are broadly classified as reference entities (ref.VESSEL for example) or data entities (data.VOYAGE for example).

Within a particular entity or table, the rows of items are called attributes. Attributes refer to the data elements that are to be saved for each entity.

The lines between tables represent linkages or relationships between entities. In the diagram above, the relationship links have been simplified to allow general depiction of a table structure. In a relational database, relationship links between tables are tightly defined at the individual attribute level and may be classified as one-to-one, one-to-many, many-to-many or as pass-through linkages.

Each entity is generally depicted with a first attribute row indicating the primary key for that entity – a unique identifier for every record in that entity. In some cases a primary key may be based on more than one attribute. The relational nature of these entities allows efficient and consistent storage of data that may be stored in multiple separate tables linked together.

When a sick animal or mortality is recorded in a specific pen on a specific day, it will automatically be linked to consignment, vessel, location and other data. Data entities are the core tables into which raw data and information is captured during normal operations, such as details on deck conditions and events (mortalities, animal treatments etc). Data entered in one table is related to all the data entered in other tables through the relational linkages between tables.

Reference entities contain pre-entered information that can be referenced or linked to reduce requirements for data entry during routine operations. For example, lists of species and breed and class of animal can be pre-defined and entered into reference tables and information about each ship (number of decks, rows, pens etc) can be similarly pre-entered.

The table structure diagram shown here does not indicate data type for each attribute. Attributes may be based defined on as text (string) records, characters (text, numbers, spaces, punctuations, etc), dates and/or times, boolean values (yes or no / true or false) or any of many different types of numeric fields.

10.5 Application functions

A variety of information is intended to be entered into reference tables prior to a voyage. It is expected that this level of data entry would occur through a computer (laptop or desktop) working either through an internet link to a central database or working on a standalone installation on that computer.

Pre-loaded information is likely to include information about loading and discharge ports (name, location, country, latitude, longitude), ship specifications, animal details (species, breed and class), exporter details, climate attributes, lists of specific diseases (disease, syndrome, species affected, etc) and information on medicines and other products or equipment that may be loaded onto the ship in preparation for a voyage (drugs, syringes, veterinary equipment and consumables).

Some of this information may be entered once and then may not change (port names and locations). Other information may need to be entered at the time of loading (medication inventory).

Multiple portable devices could synchronise with a single server at any time, allowing any number of users to merge their data prior to daily reports being generated from the server and also allowing mobile devices to be synchronised, so they always carry the most current form of the database, allowing users to look up information anywhere on the ship. This has the dual benefits of ensuring consistent data capture and allowing the user to access any prior data from anywhere on the vessel.

At loading, the application is intended to be able to receive data as an electronic file of individual animal records (based on individual animal ID records for cattle) or to accept

entered counts of groups of animals under a mob identification system (as is used for sheep).

The shipboard app (hand-held interface) is designed to allow simple wharf-side collection of data on start times (time and date) and end times for loading, and numbers of head loaded. Events (sick or injured animals) and mortalities that may occur during loading can also be recorded using the app.

Once the ship sets sail, it is assumed that the on-board application will be running as a server app on a laptop likely to be managed by the AAV without any requirement for connection to the internet. The laptop server app provides a fully functional version of the application with the added benefit that it is more suited to keystroke data entry. The laptop server app can then be synchronised to a hand-held device running a mobile version of the application.

The intention is that the AAV will carry a hand-held device on their person at all times, so that data can be entered at any time and at any location on the ship. It also allows the user to lookup an individual animal or pen/deck to review observations or entries for that animal/pen/deck.

An important part of the design of the interface is to ensure that when a user (AAV) interacts with the device to enter data as part of a routine task, that the screen interface, navigation and data entry procedures are all designed to be as easy and intuitive to use as possible and to minimise the number of times the user has to select an option or manually enter a value. Key data entry tasks should be on the main menu. Each time a user initiates a key entry task the layout should be aligned to the way users think and work through the data collection process. When an AAV approaches a dead animal to do a necropsy for example, we expect the AAV might start with recording basic information about the timing of death, location of the dead animal on board the ship and animal descriptor information (ID, species, breed, class, sex, body condition). The AAV may then record clinical signs or history, if available. Then if a necropsy is done, findings from the gross necropsy are recorded and lastly a syndrome or one or more possible diagnoses, ranked in order of likelihood. While different individuals may perform some of these tasks in a different order, the general pattern will be similar. The interface for the shipboard application needs to be designed with thought given to the way that users actually perform their day to day tasks and which pieces of data might be entered at the same time. In this way the experience that users have with the device will be aligned to the way they expect to routinely go about their job and the device will be more likely to be adopted and used.

The device is intended to be able to receive information via bluetooth or wifi linkage so that an animal RFID tag number may be scanned and imported into a record and then updated with other information such as treatments, morbidity information or post-mortem information. Animal ID information could also be manually entered with real-time look-up functionality to search through pre-loaded lists of animal ID records so that nearest matches can be displayed and selected at any time.

The AAV can then use the hand held device as the major data recording device for any information that needs to be recorded, reducing the need for transcription of data, and the associated risk of errors.

The AAV has responsibilities to submit a daily voyage report as per ASEL and the *EC (Animals) Order 2004*. The shipboard app is intended to allow collection of:

- observations per day and deck on climate measures;
- observations at any location on animal health events (any observation related to an individual animal, pen of animals or on any relevant matter such as pen or deck conditions);
- records of any treatments applied to individual animals or to pens or animals;
- records of any mortalities including post-mortem findings, if a post-mortem is completed;
- general deck level observations on measures such as faeces, respiratory patterns, heat stress, feed and water consumption and other information;
- observations about position (lat/long), sea and general climate conditions.

The hand-held device can be synchronised with the laptop server application at any time. The application will be able to automatically generate pre-defined reports such as the daily voyage report including where required generation or aggregated or other summary calculations (cumulative and daily counts of mortalities by deck and expressed by class, breed and species). Ad hoc queries and data exports for analysis using spreadsheet or statistical packages will also be possible through the laptop server application.

At a destination port, observations can be collected on time and date for start and end of unloading, counts of animals unloaded and any events (illness, injury, death or other observations) that may occur during unloading.

The server application can be used to generate an end of voyage report using a pre-defined format.

Finally, when internet connectivity is available the application can synchronise with a web server to upload all records into a secure archive. This will allow additional and more detailed analyses as required and will allow operators and relevant industry bodies with appropriate authorities to use the data either for commercial business management purposes or for whole of industry aggregate analyses and reporting.

10.6 Additional functionality

The shipboard application described in this chapter was purposefully constrained in scope to the voyage (loading – at sea – unloading) component of livestock export and to data collection requirements based on meeting daily voyage and end of voyage requirements.

During consultation with industry stakeholders in the design and discussion of prototypes, there was interest in extending the application to provide records of feed inventory and similarly for medications. The application could be extended to provide both of these services.

A brief description is provided for management of inventory of veterinary equipment and medications.

The application could be populated with reference lists of commonly used equipment, consumables and medications. When inventory is loaded onto the ship the application could be used to record what products are loaded and how much (how many units).

During the voyage, administration of any treatment to an individual animal or a pen of animals could be recorded (product, volume or number of units, any consumables such as syringe, needle, bandage, etc).

At the end of the voyage there could be a reconciliation of equipment and product inventory and the information used for assessment of voyage performance and to inform ordering practices for future voyages.

It is anticipated that there will be future development of whole-of-chain performance monitoring systems to manage QA, regulatory compliance reporting and for efficient general business management. Many of the issues and options for systems to manage performance data across the supply chain are discussed in a recent report by Perkins and Madin [78] and in a related report on QA programs by Peter Schuster. [79] There are also ongoing activities aimed at reviewing welfare outcomes in exported livestock and developing additional measures that may be recorded and reported for performance monitoring in an industry QA program.

The shipboard application would need to be modified to ensure that it can be used to collect whatever raw data measures may be required to meet QA and other regulatory reporting requirements.

A shipboard application is a discrete system that can be developed as a stand-alone application because while the ship is at sea and particularly when an AAV is moving around the decks, there is likely to be no functional internet connectivity and the system has to function as a hand-held data capture device. This is an important point because it means that a decision to progress with developing a functional shipboard application is not dependent on any decision to develop a larger, whole-of-chain system of which the shipboard app might be one component.

The prototypes described in this report have been developed with three levels of functionality:

- Web-based application that can receive and store data and provide full functionality from anywhere with an internet connection;
- Server application installed on a laptop (a stand-alone installation of the main web application that is capable of functioning as a server on a laptop without any internet connection) that allows full functionality to be maintained during a voyage; and
- A hand-held application running on a mobile device (Android or iOS) that can provide data entry and querying capability anywhere on the ship and that can be synchronised with a server application that may be running on a laptop or on a web-server.

If a larger-scoped, whole-of-chain information management system were developed for collecting and managing performance data from across the supply chain, then the shipboard app just needs to be able to communicate and share data with the larger system in order to achieve effective whole-of-chain coverage.

At the start of a voyage the shipboard app may need to be able to receive data from a whole-of-chain system, such as shipboard lists of animals and other key information. At the

[78] Perkins and Madin (2013)
[79] Schuster (2013)

end of the voyage the shipboard app needs to be able to export key data and information collected during the voyage into a whole-of-chain system.

10.7 Learnings from prototype development and feedback

Our purpose in developing prototype applications was to use a range of low cost, prototype solutions in order to demonstrate functionality to potential industry users of such a device, and capture their feedback.

This section summarises the findings from our experience in designing and developing the prototypes described in this chapter and incorporates feedback from industry stakeholders derived from hands-on testing of prototypes and from discussion of general functionality.

These points are expected to be useful along with comments earlier on the design attributes and database table structure, if a fully functional mobile application were to be developed.

10.7.1 Support for mobile application

There was good in-principle support for the concept of a mobile application using low-cost hand held devices to capture animal health and other related performance monitoring data during the voyage.

If a system is designed that is easy to use, efficient and intuitive, it is highly likely to be adopted and is highly likely to result in an improvement in the quality of collected data.

10.7.2 Functionality must not depend solely on the internet

A large number of apps can only function effectively while high speed internet access is available and live. However, on board a livestock vessel (even in port) and particularly on the livestock decks it is common for signal degradation to limit receiving of phone calls and data. Once at sea, even if internet is available on the bridge, it is likely to be expensive and restricted. The primary requirement for an “app” is that it can run completely “stand-alone”, and that it can be backed up during a voyage to a simple but specially configured computer.

This can be achieved very simply by installing a server app on a laptop. It is assumed that every AAV will have a laptop in their cabin.

While the shipboard application must be able to function independently of internet access to allow data collection during the voyage, the ability to link to via internet to a web based database remains the most effective method for managing data across the supply chain and across multiple separate voyages.

10.7.3 User interface is critical

The biggest benefit of the prototypes was the feedback on user interface. There is no question that the prototypes worked, but early testers of the device provided strong feedback on the importance of ease of use and the importance of being able to intuitively navigate around the application. Feedback was likely to reflect the fact that the export industry may not include people with the highest knowledge of apps and hand-held devices in general and this will change over time, however the issue is considered to be fundamental to development of a successful app.

- All aspects of the interface must be intuitive (including configuration). It is likely to be used by staff with a wide range of educational backgrounds working in a remote location.
- Data entry must be as simple as possible – the use of drop down lists, tick boxes etc to avoid typing is important.
- Uncluttered design is vital – low light, difficulty in keeping spectacles clean etc mean that the display must be easy to read.
- The minimum required dataset should be small. Overly onerous requirements to complete all fields will prevent user compliance.

From a design perspective this means understanding what activities an AAV will routinely perform and how they expect to be recording data and related information. This process will guide the design of the application and in particular the user interface – what menu items should be on what screen and what options/links need to be designed so that each routine activity can be achieved with minimal interaction with the hand held device.

10.7.4 The device can't be precious, but the data is

The device will (should) form the primary (and preferably only) point of data capture for most observations. This means that any damage to the device (or loss) will mean loss of data. This is no different to the traditional notebook, and in fact offers a potentially useful solution that is not available for the written notebook.

The Device must:

- be inherently robust;
- be small enough to be carried in a pocket;
- have a bright enough screen to allow visibility in direct sunlight and below decks;
- have sufficient memory and processing capacity to store data and rapidly process commands; and,
- be rapidly replaceable (low unit cost). They will get lost and broken.

The supporting database service must be:

- easy to use – requiring no more skill than to be able to turn it on and plug in the device;
- quick to synchronise data; and,
- able to share data back to devices, so that all users can have access to the full range of information about animals (ie diagnostic and treatment history) at any time.

These characteristics mean that if a hand-held device is backed up or synchronised to the laptop every time the AAV enters the cabin, then loss or failure of a device will only mean loss of data entered since the last synchronisation.

10.7.5 Custom application vs off the shelf

Our experience is that any future shipboard application will require custom development to suit the specific end users.

Any system developed through modification of existing frameworks or applications is likely to be associated with problems in user interface (ease of use) and functionality and in our view will then not be well received or widely used.

10.7.6 Leveraged functionality

There are a number of functions that may be added to a hand-held device to increase (leverage) the potential value of the device and also ensure that it is carried everywhere and used. As mobile phones started to provide more functions than just phone calls (camera, alarm clock, timer, calendar, note recorder, etc), they became more important to carry everywhere.

The current generation of multi-function devices gives a user more reasons to remember to carry such a device. Commonly available (or potential) features of such a device of value to an AAV or stockman would include:

- photographs,
- ear tag scanning and recording of RFID records,
- voice memo recording, and
- reference or resource material.

The ability to have access to one of more of the items on this list was widely appealing to different users, and their inclusion will improve the chances of the device being carried and used.

One of the issues not satisfactorily resolved during this project was the linking of a hand held device to a small portable ear tag reader for NLIS tags. The small printing of numbers on these tags and the potential delays and errors in data capture would limit the number of animal tags captured, however most researchers are in agreement that accurate reporting of animal outcomes is vital. Although data capture was possible using a Bluetooth enabled NLIS RFID scanner, at the time this report was written, it was still somewhat cumbersome. An NLIS ear tag reader would need to be carried (another device to charge, carry and use) and the ear tag reader has to be synchronised to the hand-held device to transfer an ear tag record to the shipboard application at the right time and linked to the right record. It is expected that either RFID tag readers will be developed, as smaller and more easy to use devices or that software capacity will be developed so a hand-held device can be used to scan and read RFID tags.

10.7.7 Security and privacy

Industry stakeholders have repeatedly raised concerns of commercial sensitivity and privacy and have tended to react negatively to any discussion of systems that might involve a centralised database of any form. Such concerns are valid, however it is our opinion that security can be managed at whatever level industry wishes to achieve.

There are many perspectives to any discussion about collection and management of sensitive data.

Almost everyone already uses internet systems to house and manage sensitive data with various levels of security to control access and ensure privacy. Examples include: internet banking, company records, state and national animal health records and many others. Any form of internet access involves risk management, although it can be managed.

The first demonstrated versions of the EpiCollect system were built with a single, central, shared database. Within this, records were stored by vessel and voyage and consignment and exporter and a variety of other ID fields. It is possible to implement specific security

controls in a shared centralised database, such that each user has their own access (user name and password) and can be restricted to access only those records that are relevant to their username. Different users may have different levels of authority (ability to read but not change any record, ability to change data or enter new data, ability to authorise others and so on). The levels of security implemented in shared systems can be made as complex and powerful as are required.

We have also developed relational databases where the data tables (back end of the database) are separated from the user interface and functionality (front end of the database). This allows a common or shared interface (front end) that can be maintained and updated as required, that could be web-based but that does not store any sensitive data. Individual users (exporters for example) could then install their own individual copy of the data tables (the back end) and store their own data and no-one else's data in these tables. Users could then manage their own levels of internet access to their own data tables. When a common or shared front end is linked to a specific back end then the full functionality of the application is enabled but only for the specific user's data.

Finally, it is possible to develop a software application and associated server (a full working system) and have each separate user install their own separate copy. This approach loses much of the benefit of central data storage and requires potentially more investment in product maintenance and upgrades since they may need to be updated across multiple individual users and each version may need more development to retain backwards compatibility.

10.7.8 Flexibility vs specificity

Our starting brief was to build a system that would meet current regulatory reporting requirements based on the daily voyage report and end of voyage report.

The data requirements for these regulatory reports also offer considerable strategic and business benefit to the export industry through QA and to individual exporters for fine tuning business operations to improve performance and profitability.

Any system needs to be sufficiently flexible to allow industry needs to be achieved. If performance monitoring and reporting requirements change then the system needs to be flexible enough to be capable of changing to meet future needs.

10.7.9 Real time reporting

One of the most significant benefits of the shipboard application is the ease with which regular reports (both mandatory and customised) can be compiled. Essentially, as long as the data from devices has been synchronised with the database, any report should require only the push of a button. Such reports could include:

- daily voyage reports;
- end of voyage reports;
- company specific reports;
- autopsy reports;
- animal histories;
- treatment summaries; and
- pharmacy inventories.

Regardless of the type and content of the report, the most common user question about using a portable device to record shipboard data was ***what's in it for me?*** The prospect of being able to push a button to produce simple, accurate reports was well received.

This question also extends to industry decision makers (exporters, ship owners) who may make be looking for return on investment in some tangible way (reduced costs – including compliance costs) and improved performance (reduced morbidity and mortality).

10.7.10 Integration with other systems

The voyage requirements for data collection and monitoring are discrete and defined. They start with loading of livestock onto a vessel and end with unloading at a destination port.

It is possible to develop a shipboard application and web-based server independent of any broader initiatives associated with whole of chain performance monitoring.

If and when whole of chain performance monitoring is implemented, then the shipboard application just needs to be able to communicate at both ends (upload loading plans or lists prior to the voyage starting and download voyage data and unload lists when the voyage ends) to allow effective integration across the chain.

There is sufficient knowledge and technology to build a shipboard system now.

If a broader, whole of chain performance monitoring system is developed in the future, the shipboard application will be able to function as one component within that broader system.

10.7.11 Development steps

The following general steps have been identified by the project team in consultation with the commercial developer as an indication of the likely process should a decision be made to complete development of a fully functional shipboard application.

1. Revisit required outputs, work flows, data inputs and database table structure and relationships.
2. Develop web application server for data storage and user management.
3. Develop a prototype mobile application that runs through a web interface for testing and refinement. This allows development to be modified based on ongoing review and testing until assumptions and functionality and interface layout have been tested.
4. Develop a process for pilot development and testing/review.
5. Develop the prototype application as a fully functional, mobile app on a selected (Android or iOS) platform.
6. Develop import/export interfaces for both server and mobile applications and analytical and reporting functions for the web server.
7. Launch beta version of the application (web + mobile) for review by a pilot group.
8. Develop application support protocols and roadmap.
9. Develop website and public information.
10. Incorporate pilot feedback into final product development.
11. Launch application for mainstream usage.
12. Manage ongoing support, maintenance and development.

10.8 Conclusions

The project team decided at the beginning of this project that it would not be feasible to develop a functional app for use during the main data collection part of the project. A paper-based recording system was developed based on discussion with stakeholders and modified through the course of the project as a result of feedback from users. Our view at completion of the project is that this approach was the right decision for the project.

The project team remained committed to the long term view that a web-based information management system coupled with a mobile application running on a hand-held device offers major advantages in collection of performance monitoring data during a voyage and contributing more broadly to industry QA, regulatory compliance and good business management. A well designed, mobile shipboard application has the potential to eliminate paper records during the voyage and greatly improve data quality and completeness and usefulness for the industry.

In developing and testing prototype apps we were aiming to test feasibility of different approaches and fine tune design specifications. We felt that it was necessary to first review options for development platforms and obtain feedback on aspects of user interface and design before any attempt to produce a fully functional system. We also recognised that there is considerable change being considered in the regulatory framework including the development of a QA program and possibly different welfare and performance measures.

Our experiences have substantially contributed to an understanding of the design requirements for a system if it were to be developed. We have also confirmed that there is interest and a willingness amongst industry stakeholders and particularly AAVs in using hand-held devices to more efficiently capture and manage voyage animal health monitoring data.

We are very confident that the only effective approach to developing a shipboard application is through custom development and not through modification of an off-the-shelf software product or application framework.

During development of a prototype system we received feedback from commercial app-developers that the cost of developing a system that met the design and functionality characteristics as described in this report, would be more than $50,000 and may exceed $100,000. It is not possible to accurately define costs, because they are directly dependent on final design and function, however this estimate does provide a ballpark indication of possible development costs. There would be additional costs associated with maintenance, support and further development on an ongoing basis.

The information presented in this report is expected to be of direct use in defining specifications for development of a shipboard application, if this is supported.

11 Cost of disease

Results on occurrence of disease from earlier sections of this report were combined with data and information from a variety of other sources to generate estimates of costs of mortality, both overall and for the two major causes (BRD and musculoskeletal injuries).

Estimates on general occurrence of BRD and other conditions in land-based feedlots and associated costs were drawn from recent MLA reports. [80]

Value estimates for animals free on board (FOB) were based partly on recent reports and allowing for increased compliance costs based on recent information released by the Commonwealth Department of Agriculture. [81]

Table 43 shows summary data for exports to the Middle East and North Africa (MENA) and to South East Asia, expected mortalities based on findings of this report and estimated costs of losses occurring on voyages that are due to deaths from bovine respiratory disease (BRD) and muscular injuries, the two major causes of death identified in this study. Mortality estimates as a percentage of cattle loaded were derived from a combination of detailed findings from this study and longer-term, whole-of-industry averages from retrospective data.

Table 43: Estimated annual mortality and industry costs for bovine respiratory disease (BRD), muscular / injury conditions, presented for Middle East and North Africa (MENA), SE Asia, combined total for MENA and SE Asia, and finally a total for all cattle exports. FOB = free on board.

Parameter	MENA	SE Asia	Combined	Total exports
Annual number of cattle loaded for export	150,000	600,000	750,000	850,000
Mortality, all causes as % of cattle loaded	0.33	0.08	0.13	0.12
Mortality due to BRD as % of total mortality	55	55	55	55
Mortality due to muscle/injury as % of total mortality	15	15	15	15
Value of animals FOB	$1,000	$1,000	$1,000	$1,000
Outcome estimates per year				
No. deaths due to BRD	272	264	536	578
No. deaths due to muscular/injury conditions	74	72	146	158
Number of deaths - total	495	480	975	1050
Cost of deaths per year				
BRD deaths	$272,000	$264,000	$536,000	$578,000
Muscular / injury deaths	$74,000	$72,000	$146,000	$158,000
Total deaths	$495,000	$480,000	$975,000	$1,050,000

Numbers of cattle exported to different destinations were derived from industry data since 2010 and drawn from Section 9 and industry statistics released by Livecorp.

[80] Perkins (2013); Barnes et al. (2014)
[81] Hassall and Associates Australia (2006); Clarke, Morison, and Yates (2007); Livecorp (2013)

The most detailed data on mortality estimates were available for MENA voyages and these were based on information presented in this report. We reported that 50% of deaths were attributed to BRD and that there was evidence of BRD in another one third of animals dying from other reasons. Industry data and Commonwealth mortality investigation reports indicated that reportable mortality events occurred on about 2% of long haul voyages and less than 1% of short haul voyages, and that BRD was a noted cause of elevated mortality on some but not all of these voyages. These additional opportunities for BRD to contribute to more deaths were modelled to produce a final overall estimate that BRD is contributing to 55% of all deaths per annum at the current time.

The estimate that muscular or injury conditions caused 15% of deaths was taken directly from Section 9 of this report.

Separate mortality estimates were produced for MENA and SE Asia voyages, because our findings indicated that MENA voyages had a higher mortality percentage (and rate) than SE Asia voyages. The column labelled ***Combined*** in Table 43 is the total of MENA and SE Asia estimates in combination. The final column is an attempt to produce whole of industry estimates. The total count of cattle exported (850,000) and total percentage mortality were drawn from Livecorp estimates and industry publications from the last few years.

Free-on-board value for feeder / slaughter cattle was drawn partly from Livecorp statistics for the 2012-13 year and prior industry economic analyses that dated back to 2006 and 2007, and was increased to allow for a relatively large rise in Commonwealth compliance costs that were implemented on exporters as of 1 July 2014.

The findings indicate that BRD mortalities alone are costing the industry more than $0.5 million per year and costs of all mortalities combined are about $1 million.

These findings do not represent the total cost of BRD, because they do not incorporate any marginal costs associated with morbidity and associated costs in treatments and in lost value, as animals do not grow as well or they take additional time to reach market specifications. Our estimates also do not include any economic losses due to morbidity or mortality that may occur post-discharge, because this data was not available for our study.

Our project had intended to collect limited data on morbidity during export, however this was not able to be achieved. Data from two Australian reports [82] was then used in conjunction with recent information from a USA study [83] to generate approximate estimates for costs associated with morbidity for BRD. The two most recent and relatively large scale surveys of Australian land-based feedlot health have produced very similar estimates for BRD morbidity and mortality with 0.7-0.8% of feeder cattle dying from BRD and 18-19% of animals being pulled for BRD (removed from a feedlot pen and presented to a hospital pen for examination and treatment). [82] Barnes et al (2014) then indicated that 75%, 20% and 10% of BRD pulls were treated once, twice or three times, respectively for BRD. Animals diagnosed with BRD are known to have reduced growth, feed conversion and final carcass value, though it is difficult to identify clear and well justified estimates for the reduction in nett value in animals that have been affected at some level by BRD. Australian survey results indicated that feedlot operators felt that animals receiving treatment for BRD had lost values ranging from

[82] Perkins (2013); Barnes et al. (2014)
[83] Cernicchario et al. (2013)

$20 to more than $200. Cernicchario [83] reported reductions in net value for animals based on the number of treatments they had received for BRD and these values have been adapted for this report. Animals receiving three or more treatments were likely to have serious and ongoing complications associated with BRD while animals receiving fewer treatments had a small reduction in nett value.

Including these additional assumptions allowed us to produce general estimates of total costs for BRD in export feeder cattle (see Table 44).

When treatment costs and losses in value are incorporated into overall costs, the annual impact of BRD in the population of exported cattle is estimated at $1.4 million per year.

If this cost is divided by the total number of cattle exported, it produces a cost per animal exported of $1.63.

Table 44 provides estimates of annual economic costs for losses from bovine respiratory disease (BRD) during the voyage that include components due to mortalities and morbidities. It is important to note that these estimates do not include losses that may occur after discharge.

Table 44: Estimated annual costs of voyage-related BRD in export cattle including mortality and morbidity

	Count (n)	unit value ($AUD)	Total value ($AUD)
Total cattle exported	850,000		
Total deaths due to BRD	578	$1,000	$578,000
Effects of BRD morbidity			
Treatment costs			
Estimated number of animals treated once for BRD	11,500	$20	$230,000
Estimated number of animals treated twice for BRD	2,500	$40	$100,000
Estimated number of animals treated 3 times for BRD	1,500	$80	$120,000
Nett loss of value in animals treated for BRD			
Animals treated once or twice	14,000	$15	$210,000
Animals treated 3+ times	1,500	$100	$150,000
Total cost			$1,388,000
cost per animal exported			$1.63

These findings highlight some of the complexities associated with discussion over the impact of the condition and options for risk management.

BRD was the major cause of death in export cattle in our study.

We used assumptions about morbidity based on land based feedlot research because similar data was not available for export cattle. It is possible that the morbidity effects of BRD in export cattle may be different to those in land based feedlots, however we are generally

confident that there will be cattle affected by BRD that do not die and that incur costs associated with treatment and with subsequent reduction in performance and / or market value. Further work is needed to clarify these impacts and refine cost estimates.

Our results do need to be interpreted in light of the total number of animals exported. The export industry is operating at an annual percentage mortality that in recent years has been between 0.1 and 0.15% of cattle loaded. When BRD mortalities are expressed as a percentage of cattle loaded, BRD mortalities account for an estimated 0.07% of cattle loaded. BRD morbidities are likely to involve a much higher percentage of cattle loaded – as many as 1 to 2% of cattle loaded, depending on assumptions about morbidity.

The cost per healthy animal loaded provides an economic break-even estimate for risk mitigation measures that are applied to the entire loaded cattle population and that are aimed at preventing the disease of interest.

A preventive therapy such as a vaccine that was applied to the entire healthy export cattle population may represent a net cost to the industry if marginal treatment costs (product plus consumables) exceed the per-animal cost of the disease being treated or prevented. This is because the preventive measure (vaccination) must be applied to the entire population in order to mitigate risk of disease and death that may affect less than 1% of the population.

However, there are non-economic considerations that may influence a decision to apply a particular intervention. Animal health and welfare concerns may support the development and implementation of risk mitigation strategies, even if they represent net cost to the buyer and / or vendor. This is because risk mitigation measures that reduce perceived health and welfare impacts of disease may have important benefits related to public perceptions about the industry and social licence. These benefits may be difficult to quantify in economic terms.

Such intangible benefits can have major impacts on longer term industry sustainability and may justify industry expenditure on measures that exceed simple cost-benefit measures.

If preventive measures can be targeted based on risk and applied to a limited and high-risk segment of the export population then this may achieve much of the benefit (reduction in morbidity and mortality), while avoiding unnecessary costs, because costs are incurred only for some animals and not for all animals.

This requires additional information on understanding risk factors particular for animal and mob-level risks such as species, sex, age, breed and so on. We were not able to collect detailed data to assess these factors with confidence. Tropical breeds have been shown to be at reduced risk of BRD under Australian conditions [84] and this may be partly explaining elevated risk seen in our results for cattle loaded in southern ports. This information may indicate that measures such as vaccination may be most cost effective in *Bos taurus* cattle and less so in tropical breeds.

There may also be differential risk associated with time of year (month or season) but caution is urged in interpreting these findings, as time of year is also related to animal density through assembly depots and associated stressors that are known to be associated with increased risk of BRD. It may be for example that the elevated mortality risk described

[84] Barnes et al. (2014)

in this project for southern cattle in Summer may in fact be the result of increased animal mixing and density and not directly due to seasonal factors.

It is likely that a combination of general and targeted risk mitigation measures may be appropriate for reducing morbidity and mortality risk in export cattle. General measures may be based on management practices to reduce stress and co-mingling. Specific measures may involve the use of more targeted interventions that may be associated with additional costs, such as vaccine or medication. If there is available information to identify higher risk groups of animals, then interventions may be applied to those groups and not to all animals, as a way of ensuring cost-effective risk management.

12 General discussion

This project originated in part from concerns over respiratory disease as a cause of mortality in cattle exported from Australia to the Middle East. An initial scoping study was completed to assess design options for a project to investigate causes of death in exported cattle and more specifically respiratory disease, as a specific cause of interest. This project was then designed and implemented as a result of that process.

This project has been a large, complex and innovative undertaking.

A key feature of this project was the collaborative and innovative approach to involving exporters and their staff -- particularly AAVs -- in the process of collecting and contributing research data and information.

This approach ensured that we could enrol a larger number of voyages and collect information from a larger number of mortalities than would have been possible if all data collection had been undertaken by members of the research team.

This approach also meant that we had to limit our data collection procedures to priority activities, because we were asking AAVs to undertake activities and complete forms for the research project while they had to complete all their routine day-to-day activities as well.

A second key feature of this project is that it has contributed to development of a substantial body of resource information and training material about optimal management of animal health and welfare during export and investigation of morbidity and mortality.

Finally this project has delivered against all of the objectives in the terms of reference and has advanced our understanding of causes of mortality in export cattle.

12.1 Causes of death

This project has described the major causes of death in cattle exported from Australia to long haul destinations including the Middle East (Israel, Saudi Arabia, Bahrain), North Africa (Egypt, Libya), Turkey and the Russian Federation (Russia). The findings are likely to be applicable for all long haul cattle voyages.

The most important cause of mortality in the voyages studied was bovine respiratory disease (BRD), accounting for 50% of deaths that were investigated. This was followed by

musculoskeletal and injury-related conditions that were responsible for another 15% of deaths.

It is also noteworthy that BRD was identified as a concurrent disease in one third of animals where the primary cause of death was assigned to any condition other than respiratory disease. Our findings do not indicate whether BRD in these cases may have preceded other diseases, i.e. whether BRD may have been the primary disease and increased the predisposition of an animal to another disease that ultimately killed the animal, or whether BRD in these animals may have been a secondary complication of the other conditions.

The two most important causes of death accounted for 65% of all mortalities.

Other causes of death were responsible for 1 to 6% of mortalities, representing relatively minor contributions to overall mortality counts.

Ketosis (inappetence) accounted for 6% of mortalities, but was observed mainly in pregnant breeder animals and may not be an important cause of death in feeder / slaughter animals.

Septicaemia was the primary cause of death in 5% of mortalities.

Enteric disease (diseases of the gastrointestinal tract) was the primary cause in 5% of mortalities.

Heat stroke was identified as the primary cause of mortality in only 1% of deaths.

The cause of death could not be determined in 16% of cases.

12.2 Diagnosis of causes of death

Our findings suggest that BRD can only be diagnosed effectively during a voyage as a cause of death using a combination of ante-mortem observations and gross necropsy. Clinical observations of sick animals (ante-mortem records) are not sufficient to identify cases of BRD with confidence. This is an important finding and is likely to reflect difficulties in detecting clinical signs and inability to physically examine individual animals during the voyage, the fact that some animals may have severe disease and not show much overt clinical signs and that the clinical signs that are displayed may not be very specific for BRD and may be due to any of a number of different underlying diseases.

Musculoskeletal conditions and injuries can be effectively diagnosed as a cause of death using ante-mortem observations. This is likely to be because affected animals display obvious clinical signs such as gait abnormalities, reluctance to move or inability to stand, that can be detected readily by general visual inspection of pens. Gross necropsy remains useful even for animals with visibly noticeable musculoskeletal conditions because gross necropsy will determine whether other conditions such as respiratory disease may have contributed to death in an animal with a musculoskeletal injury.

Other conditions can only be reliably diagnosed by gross necropsy.

There are constraints during the voyage associated with the general scale of decks and pens and animal numbers and difficulties in moving, restraining and examining or treating individual animals. These constraints mean that it is difficult to reliably record specific clinical

observations on individual animals or to detect development and progression of clinical signs within individual animals. These same constraints are present in land-based feedlots.

As noted in the *Veterinary handbook for the live export industry* [85], it is possible to generally inspect large numbers of animals using inspection methods detailed in the handbook. These are based on visual inspection of animals in pens and will reliably detect non-specific signs that are indicative of serious disease, particularly when there are multiple animals that are affected. For musculoskeletal and injury conditions these visual inspection methods may be sufficient to detect individual animals and identify the general disease category (musculoskeletal disease or injury) with confidence. Visual inspection of animals in pens will not reliably allow diagnosis of specific diseases such as BRD, because such methods detect non-specific signs that may be common to many different diseases.

It is not practically feasible nor necessary to have every single animal that dies during a voyage be subjected to a detailed and comprehensive necropsy with a range of biological samples and digital images collected for subsequent examination and testing in a pathology laboratory to determine the cause of death.

However, It is important that AAVs conduct gross necropsy examinations of animals that die during a voyage where possible. For voyages that do not have an AAV on board, the senior stockperson should receive sufficient training to be able to conduct a necropsy with confidence and describe gross changes in key organs, particularly lungs and selected abdominal organs.

We have developed simplified procedures during this project for conducting gross necropsies on a subset of dead animals to ensure maximal information is derived from necropsies while also minimising the time required to perform necropsies. These procedures have been described in the Veterinary Export Handbook (W.LIV.0252) and were applied during the research activities for this project, however they are not currently described in the *Veterinary handbook for the live export industry*.

It is recognised that these procedures may not be sufficient to provide maximal likelihood of identifying all causes of death. In addition, not every dead animal is likely to be necropsied.

The use of targeted or simplified necropsy procedures will allow reliable identification of the major known causes of death in export cattle. In those voyages where larger numbers of mortalities occur, this approach will ensure confidence in distinguishing the relative contributions of major causes of death, such as BRD vs heat stroke vs enteric conditions or musculoskeletal conditions.

The key principles are as follows:

- Gross necropsy should be focused on identifying the major recognised causes of death occurring during export voyages: BRD, musculoskeletal / injury, inappetence, enteric disease, heat stroke.
- Where multiple deaths occur from the same general disease syndrome and around the same time, (within a few days) then gross necropsy should be done on several (3 or more) fresh carcasses to try and establish cause of death.

[85] Jubb and Perkins (2012)

- If the same syndrome continues to be associated with deaths over subsequent days, then additional gross necropsies can be conducted on fewer representative animals (one or two animals per day) in order to confirm the gross cause of death.
- If animals die with different signs or if initial gross necropsy does not identify a cause of death, then a more comprehensive necropsy may be conducted in an attempt to identify the cause.
- Digital images of standard views at necropsy and of any lesions provide an option that may allow peer review at a later date and discussion of possible explanations and classification into causes.
- If there are concerns that deaths may be occurring with signalment, signs or gross necropsy changes that are inconsistent with the recognised major causes of death, then consideration should be given to further investigation including preparation on the same or subsequent voyages for collection of biological samples that can be imported back into Australia for examination by veterinary pathologists.

It is suggested that consideration be given to incorporating the use of a targeted or simplified gross necropsy protocol into future revisions of the *Veterinary handbook for the live export industry*.

12.3 Pathogens associated with respiratory disease

This project developed and applied quantitative PCR (qPCR) methods, in conjunction with histology and serology, on selected samples to detect pathogens associated with BRD and to improve our understanding of the epidemiology of BRD in export cattle. This is the first time that these methods have been applied in large scale testing on samples from commercial export voyages.

The qPCR techniques developed during this study provided sensitive methods (diagnostic sensitivity of <10 gene copies for most organisms) for the detection of nucleic acids from viruses and bacteria of interest in swab and tissue samples:

- Viruses of interest:
    - Bovine coronavirus (BCoV, Betacoronavirus 1);
    - Bovine herpesvirus 1 (BoHV-1);
    - Bovine viral diarrhoea virus (BVDV);
    - Bovine respiratory syncytial virus (BRSV);
    - Bovine parainfluenza virus 3 (BPIV-3); and,
- Bacteria of interest:
    - *Histophilus somni;*
    - *Mycoplasma bovis;*
    - *Mannheimia haemolytica;* and*,*
    - *Pasteurella multocida.*

One or more pathogens were detected in two-thirds (130/195) of animals from which lung samples were collected.

There was a significant correlation between detection of each of the four bacteria in lung samples and the presence of histologic evidence of pneumonia, confirming that the four bacterial pathogens were all involved at some level in cases of respiratory disease.

All of the viruses were detected in samples, though at lower rates than for bacteria, and there was no statistical association between detection of any of the viruses and presence of histologic evidence of pneumonia.

These findings support our understanding of the different roles that viruses and bacteria play in the pathogenesis of BRD. Viral infection often starts the process and viruses are primary pathogens that damage the respiratory tract and may also inhibit the immune system, thus facilitating secondary invasion by bacteria that may go on to cause a fatal bacterial pneumonia, by which time the virus(es) may no longer be detectable. [86]

Our detection of BCoV in BRD cases is the first time that BCoV has been demonstrated in live export cattle and only the second time that BCoV has been reported in Australian cattle following a recent report of detection associated with an outbreak of respiratory disease in beef feedlot cattle in eastern Australia. [87]

We detected BCoV in lung samples and nasal swabs from cattle that died and in 40% of nasal swabs from cattle in the assembly depot. An association between presence of BCoV in nasal swabs and elevated risk of BRD and reduced performance has been reported in some studies of feedlot cattle [88], while others have found no such association. [89]

It is our view that BCoV is likely to be important in the pathogenesis of BRD in Australian live export cattle.

BVDV was detected in lung samples and nasal swabs collected from animals that died during voyages. In addition BVDV was detected in 3% of nasal swabs in pre-export assembly depots and the 60% of animals tested in assembly depots had circulating antibodies against BVDV. This meant that 40% of animals were considered to be susceptible to BVDV infection.

To the authors' knowledge, this study is the first time that BVDV has been detected in individual animal nasal swabs from naturally exposed cattle with or without clinical signs of BRD.

Disease testing requirements for export cattle are based on bilateral agreements between the importing country and Australia. Our findings suggest that current protocols for BVDV may not prevent BVDV circulation amongst animals in the assembly depot and on board ship.

The presence of susceptible animals, circulating virus and the known association between BVDV infection and subsequent development of BRD suggests that BVDV is continuing to pose a risk for BRD morbidity and mortality during export voyages.

The significance of BoHV-1 and BRSV infections is difficult to determine. These viruses were isolated infrequently from animals that died during voyages and neither virus was significantly associated with deaths due to respiratory disease. In the pre-export assembly depots the nasal prevalence of both viruses was very low (~1%). To the authors' knowledge, this is the first time that BRSV has been detected in nasal swabs from beef cattle over the age of 4 months. Paired samples nine days apart in the assembly depot suggested that there was circulation of BRSV amongst animals in the assembly period. BRSV may be contributing to BRD morbidity during export voyages.

[86] Panciera and Confer (2010); Taylor et al. (2010)
[87] Hick et al. (2012)
[88] Lathrop et al. (2000); Storz et al. (2000); Thomas et al. (2006); Fulton et al. (2011)
[89] Cho et al. (2001); Hasoksuz et al. (2005)

The seroprevalence for BPIV-3 (87%) in pre-export testing was the highest out of the four viruses tested. This, combined with a low nasal prevalence and lack of evidence for an association between BPIV-3 and respiratory disease in Australian live export cattle suggests that BPIV-3 may be a common infection in young Australian cattle, but that it is not likely to be an important cause of BRD in Australian live export cattle. This is because cattle may be likely to have been exposed as younger animals and to be recovered and immune by the time they are exported.

The four bacteria of interest were detected in nasal and lung swabs from animals that died during voyages and in nasal swabs collected from cattle in the pre-export assembly depots.

Caution is required in interpreting nasal swab detection of bacteria, since all four bacteria can be found as commensal organisms in apparently healthy animals, as well as causing respiratory disease as primary or, more commonly, secondary disease agents.

All bacteria were significantly associated with histological pneumonia in animals that died of BRD. In addition, detection of three of the four (*M. bovis, M. haemolytica* and *P. multocida*) in nasal swab samples was significantly associated with pneumonia and with respiratory disease as the primary cause of death.

Our finding that there was a relatively higher prevalence of *P. multocida* compared to *M. haemolytica* in both voyage and pre-export samples supports recent reports that *P. multocida* may be rising in importance as a cause of BRD. [90]

In the pre-export assembly depot, at least 1 of the bacteria of interest was detected in up to 42% of animals, and 1 or more viruses and concurrent bacteria were detected in 38% of cattle. The prevalence of *M. bovis* and *M. haemolytica* increased significantly between entry to the depot and retesting approximately 1 week later. This increase is likely to be due to a combination of stress-induced proliferation of commensal bacteria and transmission of bacteria between animals.

The methods we developed for collecting, storing and analysing biological samples to detect pathogens were innovative and effective and have increased our understanding of the epidemiology of BRD in export cattle and the pathogens most likely to be involved in severe disease and death. These methods have the potential to be useful in further studies of infectious diseases in export animals both here and overseas.

12.4 Describing patterns of mortality in export cattle

A range of analyses were conducted on industry data derived from project activities and from other sources, including in particular the Shipboard Mortality Database (SMDB) and the NLIS database for Western Australia.

Advanced statistical modelling was applied to aggregated datasets to describe long term patterns in mortalities in export cattle. Where possible analyses were conducted using mortality rate as an outcome (deaths per 1,000 cattle-days), which allowed direct comparisons to be made between voyages of different durations.

[90] Rice et al. (2007); Welsh et al. (2004)

Voyage mortality percent has been the major method for reporting mortality in export livestock. It is simple and easy to calculate and does provide an overall measure of mortality as a percentage of cattle loaded. A major problem with voyage mortality percent is that it does not account for variation in voyage duration.

There are distinctions in purpose for different measures of mortality that need to be understood. For regulatory purposes it may be appropriate to maintain reliance on a simple, easily understood summary measure like the voyage mortality percentage. The measure is in fact described in regulatory requirements and does provide a voyage measure of performance.

The problem with voyage mortality percentage is that it does not incorporate any adjustment for voyage length. There may be benefit in reporting both types of measures since this will allow identification and distinction of short voyages that have a relatively high daily risk of mortality, but a low overall mortality percentage, because the daily mortality risk is operating over fewer voyage days, and longer voyages that have a relatively low daily mortality risk, but still have a relatively high voyage mortality percentage solely because of the length of the voyage.

For detailed scientific analyses aiming at combining data from many different voyages to compare mortality risk and identify those drivers that may be influencing mortality risk, voyage mortality percentage is not appropriate and it is necessary to use a true incidence rate measure of mortality, such as voyage mortality rate or daily mortality rate. These measures allow direct statistical comparisons of different voyages and of drivers that may be operating across voyages to modify mortality risk (season, month, breed, age, weight, sex, pre-export vaccination or treatment, ship factors, ocean conditions and other factors).

There were associations between mortality rate and various explanatory variables including year, port of loading, destination region and month of year.

Our findings indicated that mortality rate has progressively dropped over time and that mortality rates in the most recent period (2010-2012) were generally lower than in previous time periods.

Mortality rate was higher for voyages to the Middle East and North Africa than for voyages to SE Asia, NE Asia and SE Europe. Voyages to NE Asia and SE Europe may have had more breeder animals and these voyages may involve differences in animal selection and management that contribute to lower mortality risk.

Mortality rate was higher in Summer months, with this pattern most evident in cattle loaded in southern ports and destined for SE Asia. In contrast, there was little variation in mortality rate over the course of the year for cattle loaded in northern ports and destined for SE Asia.

The same pattern was seen in southern loaded cattle exported to the Middle East and North Africa.

We have also described for the first time, the pattern of daily mortality rate over time during export voyages. Daily mortality rate is not constant during the voyage and rises progressively from the start of the voyage to a peak at around week four of the voyage and then declines. This pattern is remarkably similar to plots of mortality rate over time derived

from land-based cattle feedlots in Australia, which also show a progressive rise over time to a peak at around week four post induction of cattle into the feedlot. [91] The land based feedlot mortality pattern can be attributed to the influence of BRD primarily since BRD is the biggest cause of death in feedlot cattle.

The current project did not collect detailed data on BRD risk factors in export cattle. This was largely due to difficulties in collecting such data and related information, given the design constraints of the project and our reliance on industry workers for much of the data collection. Nonetheless, our findings indicate that the epidemiology of BRD in export cattle may be very similar to that described for land-based feedlot cattle and may be driven by pathogen exposure associated with co-mingling around transport to the assembly depot and during assembly depot aggregation of animals prior to export. A range of other causal factors associated with animals, pathogens, management and the local environment are then likely to influence exposure of animals to pathogens, development of infection and disease and risk of mortality.

There are likely to be particular causal factors that operate on export vessels and that are not present in land-based feedlots. These include factors such as ventilation and air quality in closed decks, accumulation of waste products (faeces, ammonia, etc) in pens during the voyage and effects associated with sea conditions and climate (temperature, humidity) during the voyage. These factors may act as modifiers in a causal web where initial exposure and infection risk associated with co-mingling in the assembly period and early part of the voyage is likely to be essentially the same as the causal web operating for BRD in land-based feedlots.

There are two important conclusions to be made concerning BRD epidemiology in export cattle that arise from the findings of the current project.

The first is that some caution is required to avoid over-interpreting the findings from this project because of limitations in design and difficulties in identifying specific drivers of BRD occurrence and severity under export conditions. A logical consequence of this is to make the recommendation that further research is required to investigate key drivers of mortality risk with a particular focus on BRD in export cattle. However, it is also important to consider this recommendation in light of return on investment and to design approaches that build on those used in this project where research activities are embedded into routine business operations. This is discussed further in later sections of the discussion and in the recommendations.

The second conclusion is that the findings of the current project provide convincing evidence that BRD in export cattle is very much the same disease caused by the same pathogens as BRD in land-based feedlot cattle. While we are lacking detailed data to assess causal factors, we can and should embrace research findings and practical experience about BRD derived from Australian cattle feedlots to identify and implement risk management strategies to reduce BRD occurrence and severity in export cattle.

[91] Perkins (2013)

12.5 Mitigating BRD risk in export livestock

The live export cattle supply chain has many features in common with management of cattle in feedlots. This study suggests that the pathogenesis and epidemiology of BRD in live export cattle is likely to be similar to that in feedlot cattle. Therefore cattle selection and husbandry strategies used to prevent and control mortality in feedlot cattle are likely to also be effective in minimising the incidence and costs associated with morbidity and mortality (due to BRD and other diseases) in live export cattle. Prevention and control strategies aim to minimise pathogen exposure, stimulate herd immunity, and manage risk factors that potentiate the occurrence and severity of disease. [92]

Mixing of cattle of unknown health status from different properties is an important risk factor for pathogen exposure and infection. The majority of cattle sent for export, particularly those on long-haul voyages, are sourced directly from their property of origin, so co-mingling at the assembly depot presents an important BRD risk.

Risk mitigation strategies such as identifying cattle suppliers based on prior performance of cattle from that supplier, yard weaning, familiarity with feedlot feeding and watering practices and vaccination against prevalent major respiratory pathogens may have application in the export industry. The land-based feedlot industry has developed strategies for backgrounding cattle that incorporate some or all of these measures and that aim to maximise feedlot performance and minimise adverse health risks. It is recognised that applying interventions at the property requires advanced planning that may be problematic for either the land-based feedlot chain or the export chain. However, it is not defensible for the livestock industry to try and argue that risk mitigation measures are not feasible because of short lead times.

The export industry has produced training material on low-stress animal handling of animals in the export chain and supports this with direct involvement in training and mentoring animal handlers in countries receiving Australian livestock by export. Many of these resources can be viewed on the LiveCorp publication pages. [93]

It should be possible to develop systems that allow risk mitigation strategies to be developed as part of routine cattle supply preparations or to be encouraged and facilitated through preferential supply arrangements or regulatory compliance and QA benefits. Moving towards these strategies would require a strategic shift in the way the industry selects and manages animals prior to export and would reinforce that industry is committed to quality and performance.

Vaccination can be used to stimulate individual and herd-level immunity. Vaccines are generally most effective if used in accordance with manufacturer guidelines and for many vaccines this may require two initial vaccine doses administered some weeks apart. This requires the same sort of planning as was discussed in the previous paragraph.

More recent developments in BRD vaccines have focused on the development of single-shot vaccines that can be administered to cattle at induction. Some of these products are administered as an intra-nasal spray and can result in rapid onset of local immunity to

[92] Perkins (2009); Edwards (2010)
[93] http://www.livecorp.com.au/publications

combat respiratory tract pathogen exposure and infection. In Australia there is now a one-shot vaccine registered for use in cattle to protect against *M. haemolytica* (Bovi-Shield MH-One®). There are also registered vaccines in Australia for use in cattle to protect against BoHV-1, BVDV and there may be additional products used under limited licence for specific situations.

In the USA and Canadian markets there are more diverse offerings of single shot beef cattle vaccine products that are directed against many of the recognised BRD pathogens. In fact one or more single-shot vaccine products can be identified in the USA or Canadian markets for all of the BRD pathogens studied in this project, with the exception of BoCV. The relevance of this is only to indicate that production of these vaccines is technically possible. Vaccines developed in other countries may not be appropriate for use in Australian cattle because of strain differences in pathogens and vaccines should not be used in Australian cattle unless there is evidence to support efficacy and safety.

It is very important to understand that vaccination against BRD pathogens has been widely applied in feedlot cattle around the world and that impacts due to BRD morbidity and mortality continue to occur. Vaccination is not a panacea that will eliminate BRD risk in export cattle.

Equivocal results in studies evaluating the impact of vaccination on BRD may be difficult to understand given that it seems intuitive that effective immunisation would be helpful. Possible reasons for failure to demonstrate beneficial impacts include variation in study design, timing of administration, failure of stressed animals to respond effectively to vaccine, and the complex causal web for BRD including the possibility of multiple infectious agents. [94]

A recent systematic review of BRD vaccine efficacy against the *M. haemolytica, H. somni* and *P. multocida* concluded that there was evidence of benefit for vaccination against *M. haemolytica* and *P. multocida,* but no benefit from vaccination against *H. somni*. [95]

In addition, there are strain variations for viruses and bacteria that may mean a vaccine available in one country may not necessarily be effective against the same pathogen in another country. Strain variation may even mean that a vaccine available in the same country is not always effective against the pathogen(s) it is intended to combat, particularly if there is more than one strain in circulation.

Mass medication has also been used as a strategy of mitigating risks for BRD in feedlots and in export cattle. There are distinctions between the administration of antibiotic to healthy animals on arrival (called metaphylaxis) and a decision in the face of a rapidly expanding epidemic of clinical cases of BRD to treat all animals in a group rather than identifying individual sick animals for treatment (called mass medication). Metaphylaxis has been applied in some groups of cattle being prepared for live export in an attempt to reduce BRD occurrence and losses during export, most recently with a long-acting product containing tulathromycin (Draxxin®). Concerns over metaphylaxis relate to cost, risk of antibiotic overuse and potential contribution to development of resistance and residues (depending on product and time from treatment to slaughter). It is possible that use of metaphylaxis may mask other suboptimal management practices.

[94] Taylor et al. (2010)
[95] Larson and Step (2012)

During the voyage, management of stocking rates, ventilation, bedding and other factors are based in part on management of risk to reduce morbidity and mortality risk from known problems including BRD.

A large longitudinal project investigating BRD in Australian feedlot cattle has recently been completed under the direction of researchers from the University of Queensland. [96] The final report from this project is currently being completed. This report will be of great interest for the livestock export industry in understanding risk factors for BRD in Australian cattle and possible preventive measures that may able to be applied to cattle being prepared for export. The work has identified protective effects (reduction in BRD risk) in cattle vaccinated with Bovilis MH™ and Pestigard™, but the effects of vaccination alone were relatively small and not as significant as a range of other management factors. [97] The main management factors that were identified as potentially being able to mitigate BRD risk were related to mixing, moving and grouping of animals in the period prior to feedlot entry. [97]

These findings reinforce the value in best practice principles for BRD risk mitigation that have been well described both in Australia and elsewhere and that rely on management factors such as reduction in stress, movements and mixing and ensuring animals have access to good quality water and feed. [98] There is growing understanding of the importance of stress [99] and associated factors such as distance travelled to a feedlot [100] and liveweight shrinkage during transport [101] in exacerbating occurrence and severity of BRD in feedlot cattle. It seems plausible that management and stress-related factors may be more important in BRD risk mitigation than vaccination status and regardless of whether vaccination is implemented against selected BRD pathogens, it will be important to adopt management strategies aimed at reducing risk as well.

A number of specific associations were noted in the current report that are related to management factors for BRD risk mitigation. Pathogen exposure risk in assembly depots is likely to be increased with increased mixing of animals from multiple sources and mixing of new arrivals with carry-over animals. Our findings and those of Hick et al [102] have flagged the likely importance of BCoV as a viral agent involved in BRD and the circulation of BVDV in assembly depots and during the voyage, despite testing strategies aimed at removing infected animals pre-export.

Any risk mitigation measure (including vaccination) should be carefully evaluated and applied only if demonstrated to have a beneficial effect through valid scientific studies. However, a lack of clear data does not mean that no measures should be adopted. Measures may be adopted where there is a reasonable expectation that they will mitigate risk, but in this situation there must be concurrent monitoring to assess impact and the ability to change risk mitigation measures if and when there is convincing evidence from valid scientific studies.

[96] Condon (2013); Barnes et al. (2014); Hay (2014)
[97] More (2002); HayHay (2014)
[98] MLA (2006)
[99] Hodgson et al. (2012)
[100] Cernicchiaro et al. (2012)
[101] Cernicchiaro et al. (2012)
[102] Hick et al. (2012)

Targeting mitigation measures to those groups that have higher risk offers a cost effective means of reducing morbidity and mortality but requires clear justification for differential management and monitoring to document efficacy.

Advances in technology and leveraging of health monitoring at the individual animal level in association with NLIS animal identification data offer an unparalleled opportunity for industry to collect data efficiently to allow monitoring of performance and assessment of efficacy of various management or treatment factors. See Section 12.10 for more detail.

12.6 Other causes of death

Musculoskeletal and injury-related conditions was the second major cause of mortality, accounting for 15% of the 215 deaths studied. A number of these mortalities were associated with conditions that did not respond effectively to treatment or management during the voyage and where the animals were euthanased at or before the destination port, because of welfare concerns.

The proportion of deaths in this category (15%) was lower than the 23% [103] and 25% [104], reported previously in studies on Australian export cattle. This is likely to be due to a combination of study methodology and factors that may modify risk such as vessel design, animal selection and animal management.

The mortalities described in this report for musculoskeletal and injury-related conditions are likely to under-represent the impact of these conditions on animal health and welfare. Morbidity due to musculoskeletal and injury-related conditions is relatively common and can be very difficult to manage and treat effectively during the voyage, because of constraints in animal handling and restraint and limitations in the supportive care options that can be implemented. Veterinarians with experience in routine non-export livestock work may not be adequately prepared for managing some of these conditions and there appears to be a need for information on how best to manage these conditions on-board ship and how to approach decision making, for example in determining whether a particular animal may respond to treatment or may require euthanasia. Efforts have been made to incorporate these topics in to the *Veterinary handbook for the live export industry* and these comments reinforce the value in this resource.

The risk of serious and potentially life-threatening consequences from initial injuries or conditions on board a ship that may heal without problem in other routine land-based environments, mean that it is important that all efforts be made to minimise injury risk and therefore prevent injury. Therefore careful attention should be paid to infrastructure design (pens, yards, races, gates) and maintenance, both in the assembly depot and on board the ship. In addition, low-stress animal handling is also very important to reduce the risk of foot and leg injuries that may result from crowding and hurried movement.

We recognise that Livecorp requires stockpersons to complete training that incorporates information and practical training in low stress cattle handling techniques. The importance of simple messages about risk management extend across the chain from pre-export

[103] Hedlefs (1988)
[104] Norris et al. (2003)

preparation and transport and handling of livestock, through to inspection and certification and management during the voyage and after arrival at the destination port.

During the voyage rough seas can lead to traumatic injury, however most cases of lameness develop secondary to injuries and abrasive floor surfaces, with a high risk of secondary infection due to the presence of progressively contaminated bedding in shipboard pens. [105] Lame or injured animals may then become reluctant to stand or move and risk being trampled or not eating or drinking. All of these events increase the risk of other diseases and of eventual death or euthanasia. Lame or injured animals are reluctant to stand, which increases the risk of their being trampled by pen-mates, and can lead to inappetence and dehydration.

The recent report by Banney et al [105] provides useful advice on cattle selection and bedding management to reduce the incidence of lameness. Further work is required to identify and implement risk mitigation strategies that may further reduce risk of injury and musculoskeletal conditions. For example, guidelines for optimal deck flooring materials and, where a bituminised aggregate flooring material is used, optimal aggregate size; and further work on bedding, deck washing and general management of pen flooring for optimal foot and leg health during the voyage.

12.7 Heat stress

Heat stroke has previously been reported as the most common cause of death in export voyages. [106]

In contrast, heat stroke was identified as the primary cause of death in only 2 animals in the present study (<1% of deaths), although hyperthermia secondary to other disease processes, for example severe bronchopneumonia and septicaemia, was recorded in an additional 9 animals.

This marked reduction in deaths due to heat stroke is likely to be due to changes that have occurred in the industry since the late 1990s as a result of increased industry awareness of risk factors for heat stress, and modifications to the selection and management of cattle prior to and during export. In addition, the livestock export vessel fleet has been upgraded, with a move away from refurbished freight vessels and car carriers to the production of purpose-built livestock carriers. This is supported by examination of mortality rates over the period 1995-2012 based on data from the SMDB.

However, heat stress events do continue to occur from time to time and may be associated with elevated mortalities, as reported in mortality investigation reports available on the Commonwealth Government website.

Our findings suggest that while improvements in routine practices and ship design and current mitigation measures appear to have reduced the risk of heat stress there is a need for ongoing effort to continue to use tools such as *Hot Stuff* and to monitor for and manage heat stress risk during export.

[105] Banney, Henderson, and Caston (2009)
[106] Hedlefs (1988); Norris et al. (2003)

12.8 Economic costs of major causes of death

Our findings indicate that BRD mortalities in cattle alone are costing the industry more than $0.5 million per year and costs of all mortalities combined in cattle are about $1 million per year.

We used estimates for morbidity rates and costs associated with BRD treatment and loss of market value that were drawn from recent Australian land-based feedlot studies to inform assumptions about possible morbidity related costs for export cattle for BRD.

This allowed us to produce an estimate of the total economic impact of BRD on export cattle that included costs due to mortality as well as costs due to morbidity (treatment costs and losses in value in surviving animals). Our estimates indicate that the annual impact of BRD in the population of exported cattle is $1.4 million per year.

If this cost is divided by the total number of cattle exported, it produces a cost per animal exported of $1.63.

The cost per healthy animal loaded provides an economic break-even estimate for risk mitigation measures that are applied to the entire loaded cattle population to prevent the disease of interest.

However, there are non-economic considerations that may influence a decision to apply a particular intervention, including for example, impacts on public perceptions about the industry. Such intangible benefits can have major impacts on longer term industry sustainability and may justify industry expenditure on measures that exceed simple cost-benefit measures.

If preventive measures can be targeted based on risk and applied to a limited and high-risk segment of the export population, then this may achieve much of the benefit (reduction in morbidity and mortality), while avoiding unnecessary costs, because costs are incurred only for some animals and not for all animals.

12.9 Veterinary handbook and related outputs

This project has delivered general improvements to systems and procedures that are being adopted and used by the industry for optimal animal health and welfare outcomes.

A project-specific handbook (Veterinary Export Handbook - W.LIV.0252) was developed as a one-stop resource containing information on diseases in export cattle, how to investigate disease, how to perform a necropsy and project specific requirements including standardised terminology for recording information about sick and dead cattle, and collection of samples and images from necropsies. The handbook included information on equipment and care and the project invested in a standardised set of equipment that was placed aboard every participating ship and maintained/replenished every time that ship returned to Fremantle for the duration of the project. This handbook was only ever intended to serve as a resource for the lifespan of the current project.

A training package was developed (training materials, schedule, list of activities, assessment criteria and template certificates) and delivered twice to ensure that all current AAVs and

many other interested parties received information on the systems and procedures outlined in the handbook. [107]

The package can be used as a resource for future training courses delivered for veterinarians and/or stockpersons. The project has also produced a professionally filmed and edited training DVD, that provides step-by-step instruction in how to perform a comprehensive necropsy, as well as a variety of additional relevant topics including personal safety and biosecurity and management of necropsy equipment. This DVD has been widely acclaimed and is sought after by a wide variety of groups (large animal veterinarians, veterinary schools and other groups) as a training resource.

During the course of this project, the project specific handbook concept was revisited and a generic *Veterinary handbook for the live export industry* was produced. This handbook includes information about general management and common diseases in export livestock (beef cattle, dairy cattle, sheep and goats) and was intended to act as a general resource for all personnel involved in livestock export. In 2014 this resource was released as an app for downloading onto Apple or Android devices (smartphones, tablets, computers). [108]

12.10 Veterinarians and stockpersons

Veterinarians (AAVs) and stockpersons have responsibilities described in legislation and associated regulations. The Secretary of the Department of Agriculture may require an AAV to accompany any export voyage as part of the AEP, but at the time this report was completed AAVs mainly accompany long-haul voyages. Where voyages are not accompanied by an AAV, the reporting obligations under regulations fall in part to the senior stockperson.

As described in Shiell, Perkins and Hewitt [109] (Section 6.5.1.2), there are occasions where short-haul voyages unaccompanied by an AAV have a reportable mortality event and these sorts of events have led to discussion of the pros and cons of requiring an AAV to accompany every export voyage.

There are several issues associated with the question of whether an AAV should be present on every livestock export voyage.

Having a veterinarian on board has specific benefits associated with veterinary skills i.e. diagnosis and appropriate treatment of conditions occurring during the voyage, and implementation of preventive strategies during the voyage to minimise risks of the same conditions occurring in other animals on the same ship.

However, there are understood to be relatively few veterinarians performing export voyage work and there may be difficulties in obtaining veterinary personnel, if there was a requirement for an AAV on every voyage.

While there may be genuine advantages associated with having a registered veterinarian on board, current practices already have experienced stockpersons performing these roles on voyages where veterinarians are not present.

[107] Perkins, Jubb, and O'Hara (2012)
[108] http://www.veterinaryhandbook.com.au
[109] Shiell, Perkins, and Hewitt (2014)

We believe that there are benefits from providing senior stockpersons with similar training and resource material to that provided to AAVs. In particular, this relates to material describing common conditions occurring in export livestock, managing livestock during export and in procedures such as performing post mortems to determine cause of death and even where appropriate collection of standardised samples for return to Australia where specialist pathologists may confirm the cause of death (accepting that importing samples back into Australia is unlikely to be a routine occurrence because of quarantine restrictions).

On voyages where there is an AAV present, the AAV would reasonably have primary responsibility for decisions concerning animal health, welfare, treatments and diagnosis of conditions including by necropsy. However, suitably trained and experienced stockpersons may be able to provide considerable assistance to AAVs under these situations, including assisting in necropsy procedures. Our experience in the current study was that some AAVs utilised stockpersons very effectively to assist in making observations on animals and also to assist in conducting necropsies.

The focus of training and involvement of stockpersons would be to support development of skills and capacity to diagnose and manage the major causes of illness and mortality on board export voyages. This is intended to complement and strengthen provision of veterinary care for animals in situations where there is an AAV present and where there is not an AAV present on a voyage.

12.11 Online systems development

More information on the topic of performance data monitoring for the livestock export industry can be found in a recent industry report by Perkins and Madin. [110]

Our focus during this project was on the shipboard component of the export supply chain. Comments in this section on online systems development are focused on the voyage, however the principles can be extended across the supply chain.

We believe there is an opportunity for industry to develop a strategic approach to online performance data monitoring systems that are owned by industry and that incorporate measures currently being collected and reported on through the Shipboard Mortality Database (SMDB) and possibly through voyage reporting.

The starting point for this concept is the premise that exporters and ships' Masters are already collecting and reporting on information and it is already being used for industry benefit. Moving to an online, industry-owned system provides industry with the opportunity to lever additional industry benefit from this information without requiring exporters or vessels to provide data or information other than what they are already providing. Industry investment is justified because of the whole of industry benefit and because of cost efficiencies in building a centralised core system that can be used by all exporters and by industry bodies such as LiveCorp.

During the course of the W.LIV.0252 project we were unsuccessful in trying to obtain copies of daily voyage reports from all voyages as a source of voyage-specific data on voyages. Daily voyage reports involve data collected from many different people on-board the ship

[110] Perkins and Madin (2013)

(stockmen, AAV, first mate and ship's Master) and it is difficult to get permission to access the report either through ship records or from the Commonwealth.

Our prototype hand-held device was developed for use by AAVs and senior stockpersons for collection of animal and pen/deck observations relating to health, illness and deaths. This represents part but not all of the relevant data and information for a daily voyage report. We focused on these functions because of the research focus of the project and because we were depending on AAVs for much of our data collection during routine commercial voyages.

The Shipboard Mortality Database (SMDB) provided the major source of data for analyses described in Chapter 9 of this report. The SMDB is collected through a completely separate process that involves interaction between Mr Greg Norman and the first mate or Master of each ship. [111]

We believe there are substantial benefits to industry from an industry-owned and operated performance monitoring system, both at industry-wide levels and for individual exporters. The major whole of industry benefits are related to market access, marketing, performance evaluation, QA and rebuilding of social licence and associated benefits from regulatory freedom that follow public confidence and social licence. [112]

We believe there are also substantial opportunities for individual exporters to benefit from an industry owned system by drawing relevant data out of the industry system and combing it separately with their own private and commercially sensitive systems to lever private commercial value and advantage.

Finally there are RD&E benefits from having these systems in place and able to contribute to RD&E strategy and to individual RD&E projects.

An important part of the strategic approach will be understanding and agreeing on what benefits this sort of system can provide to both industry and individual exporters, managing risks and risk perceptions, and adopting a gradual and step-wise development approach that maximises return on investment, has a very clear focus on industry ownership, and manages security and commercial sensitivities.

There will undoubtedly be major distinctions between a longer term vision of what a system may deliver in the future and a step-wise strategy in the short term to develop, implement and trial the first pieces of any broader performance data strategy.

We think a reasonable starting point is an online system based on the SMDB that can provide a sustainable future for the annual performance reports and that can be used to generate more timely performance measures and benchmarking as part of the transition to a whole of chain QA program with a stronger focus on outcomes. The system would presumably be owned and operated through an appropriate industry body such as LiveCorp.

The system could be enhanced over time by adding functionality including:

- Ability to store and report on measures currently reported in daily voyage reports. A starting point may be for the online system to receive daily voyage reports and manage

[111] Higgs (1989); (1990); (1990); Norris and Norman (2003)
[112] Arnot (2009); (2011)

these in conjunction with SMDB measures to produce outcome-based performance reports that go to Commonwealth regulators, exporters and to ship owners.
- Ability to collect, store, analyse and report on data from post discharge performance measures, including ESCAS compliance and risk assessment measures.
- Ability to incorporate data and information from earlier stages of the supply chain back to property of origin.

There are obvious concerns over privacy and commercial sensitivities. It is our belief that these issues can be effectively managed.

Online data security is a huge issue with more and more commercially sensitive data being stored online. Online data security is dynamic, under some level of constant threat and requires regular vigilance and investment in proportion to risk. The bigger risks to security in most situations are likely to be in the area of human behaviour through use of inappropriate passwords that can be hacked, through release of sensitive information and through inadvertently allowing malware / computer viruses etc to gain access to a computer. It is possible to manage risks very effectively through a combination of procedures and protocols (strong passwords that are regularly changed, current malware protection, simple measures and behaviours that lower risk) and through use of appropriate security in design and function of online systems.

Data stored in an online system can be securely encrypted before being uploaded and stored in password protected databases in an encrypted format. Encryption means that data stored in an online database cannot be read without first being decrypted by a user with an appropriate decryption key matched in some way to the encryption process. Measures such as two-factor authentication are increasingly being used to provide additional security for online systems. An example of this is when someone logs into their banking records using a conventional username / password and is then sent a one-time password via SMS to their mobile phone or is asked to enter a separate authentication code from a physical token. Some systems use biometrics (fingerprint scans on a hand-held device or laptop) for additional security. It is possible to incorporate recognition of a specific computer or even a specific IP address into an authentication process so that a registered user may only access the online system from a specific computer that is connected to the internet through a specific IP address. These measures require more intensive protocols, but greatly add to system security.

Data reliability (backup and ability to recover data) is generally considered to be more effectively controlled in an online environment than by having data stored on a computer or backed up onto an external hard-drive connected to a desktop computer. Online databases can be continuously backed-up to multiple servers in different physical locations providing a level of redundancy and confidence in ability to recover data that cannot be matched by any other approach. Industry standards for online backup services often mention nine-nines of data reliability, meaning that 99.9999999% of all backed-up data can be recovered at any point in time or alternatively that data can be recovered at any point in time with 99.9999999% confidence. The massively linked capacity of cloud-based systems means that point failures in multiple locations (computer failures, power failures or physical disasters such as earthquakes, fires, floods etc) may compromise individual components, however the massive redundancy in the internet means that backups are likely to be secure. Individuals can control the level of redundancy they wish to invoke and can also control which servers

they may use to house backups (many organisations and governments may insist on using servers physically located in their own country for example).

Online databases can also be designed and developed as separate but linked components. For example, a secure online database that stores commercially sensitive data may be completely separated from the computer that is used to access it. Online banking access provides an example. An individual can log on to their banking site from any computer and see all their detailed information stored in an online database. When they log off the computer has no information or data stored on the computer itself. This system means that even if a laptop fails or is stolen, it does not mean that data is lost or available to the thief and it does not affect in any way the security of the data stored in an online database.

It is also possible to design online services in a modular way with different areas being subject to different levels of security and access. Data that is less commercially sensitive and of more value to the broader organisation or industry can be housed in a more readily available and shared database (pre-competitive database). Additional data that is more commercially sensitive or relates directly to market advantage or fee-for-service outputs, can be stored in many different, privately controlled databases that may link to the pre-competitive data base for some measures.

Principles of database design underpin this general approach and explain why databases provide such efficiency and data integrity – each piece of data should be entered once at the most appropriate point for data collection and then linked to all other relevant data.

The Australian dairy industry is progressing with development of industry-wide online database services that are based on these two broad components:

- A core set of shared baseline data that is housed in a pre-competitive, industry-wide database that can be accessed by a wide variety of individuals and organisations with appropriate controls and security. These data tend to be raw measurements likely to be of interest and value to the entire industry and do not include the most commercially sensitive information. This includes individual animal identification for dairy cows, breed, birthdate, raw data on milk production and milk quality and a range of other measures. Development and maintenance of this system is maintained by a variety of funding bodies including R&D funds from Dairy Australia and additional funds from key partners.
- Third-party providers and other users can then link to the pre-competitive database and lever additional value that might involve IP and fees and higher levels of commercial sensitivity. Access is still controlled for example individual dairy farmers can access their own data and can authorise a third party provider to access their data. Individual producers may link core data to their own commercially sensitive data held in separate and more secure online systems. Breeding companies may link sire/dam/progeny records from multiple producers to manage genetic summaries and breeding values. Service providers (veterinarians, nutritional consultants, farm management consultants) may have their own proprietary software products that link data from the core database to individual producer data and then apply their own proprietary routines or analyses to generate reports for producers as part of fee-for-service operations. Producers may draw on raw data stored in the pre-competitive database and link this to commercially sensitive information in their own privately secure database systems to support business reporting and commercially sensitive decisions.

The systems being developed and trialled by the Australian dairy industry provide a potential model for consideration by other industries.

This two-component approach provides an efficient and effective way for industry R&D funds to underpin base-level capacity and system development while encouraging and facilitating third party providers and individual exporters to be innovative and develop additional commercial IP that is external to the core system and that links to the core system to access records.

12.12 Success in meeting objectives

All objectives were achieved. Nine of thirteen objectives were achieved completely. There were two objectives that were partially achieved (Objectives 3 and 13) and two objectives where the project requirements were met without requiring all activities to be completed (Objectives 4 and 5).

The project was preceded by extensive consultation with industry to ensure that the innovative approach developed for this project would be supported by industry. The approach was based on involvement of industry personnel (particularly AAVs) to perform disease investigations and particularly necropsies during export voyages. This approach required the support and commitment of exporters (who were responsible for employing AAVs) and the AAVs themselves. Industry stakeholders were very supportive of the approach and AAVs were very happy to be involved.

The systems and processes developed for this project have worked effectively to allow AAVs to perform mortality investigations and contribute information and biological samples to the research project.

Sufficient samples and information on mortalities were collected to allow description and characterisation of the most important causes of death in cattle exported from Australia on long haul voyages with a focus on Middle East destinations.

The innovative approach developed for this project was based on a careful review of pros and cons of different strategies. Previous studies have tended to have a small number of highly trained research personnel with members of the research team accompanying specified voyages to conduct necropsies and collect samples. [113] This approach was considered to severely limit the number of voyages that could be enrolled and would consequently limit the usefulness of the information. The benefit of this approach was that the data that was collected would be more likely to be complete and of a uniformly high quality, because the project team members would be highly motivated to perform all tasks as per protocol.

We chose to involve AAVs because this meant that we could enrol many more voyages and collect data from more cattle.

We recognised that the use of AAVs for collection of data would pose problems that would require careful management.

[113] Norris et al. (2003); Makin et al. (2009)

The use of AAVs for collection of research information and data provided an opportunity for variation between AAVs with respect to approach to classification / diagnosis, recording, necropsy and sample collection. We attempted to address this with development of the handbook and associated training, including provision of the DVD on how to conduct a necropsy and also by ensuring that every ship had a fully stocked veterinary kit, so that every AAV could perform all procedures they were being asked to complete. Detailed information was provided on how to package, label, and store samples, completed forms and electronic data and images for importation into Australia.

We consulted widely with exporters and AAVs about the procedures. Industry feedback from the pilot voyages indicated there were concerns that AAVs were being asked to undertake too much for the project and that project activities might interfere with the AAV's ability to complete routine tasks during the voyage. We responded by simplifying the necropsy procedures as much as possible, reducing the expectation with respect to the number of dead animals that AAVs were being asked to necropsy and generally simplifying processes to minimise the time required by AAVs to complete research tasks.

Our approach meant we had to be prepared to simplify procedures and forgo some data and information that may be of interest because we had to avoid overloading AAVs with unrealistic expectations. We had to focus on those priority activities that would provide maximal benefit for the research objectives while designing tasks for the AAV in a way that minimised their time commitment and ensure they would both complete research tasks and all of their regular daily tasks as well. Our review of AAV job descriptions and legislative requirements indicated that all of the procedures we were asking AAVs to do were already part of their routine tasks, with the exception of collection of biological samples and filling in the research specific forms we provided.

Compliance with project procedures was not uniformly achieved and deteriorated through the duration of the project.

There was a high level of interest and engagement initially and good compliance with the post-mortem requirements and completion of the ***necropsy / dead cattle report*** form and collection of post mortem samples. There was generally poor compliance with the separate ***daily health report form*** that was intended to provide simple data collection on general health and morbidity measures. AAVs did not fill in the ***daily health report*** form, perhaps because the information was similar to that being compiled each day for the daily voyage report that was sent to DAFF and because they were focused mainly on the dead animal form and on completing necropsies and collecting necropsy samples.

None of the project team anticipated the unfortunate events of 2011, that led to cessation of livestock exports to Indonesia and the implementation of ESCAS across the export industry. These events were associated with a great deal of stress and uncertainty within the industry and had an impact on industry commitment to and willingness to participate in this project.

Over the duration of the project, it became more difficult to enrol voyages into the project. In addition, AAVs on participating voyages tended to complete necropsies on a smaller proportion of voyage mortalities over time. The erosion of commitment to the project procedures was almost certainly exacerbated by delays in the time from end of voyage to completion of all pathology investigations on samples collected during that voyage. In some

cases it was several months before samples were imported back into Australia. Since this work was largely being done by a PhD student there were additional inefficiencies because field work requirements prevented timely completion of laboratory work.

As this report was prepared, the overwhelming sense was that industry has strongly supported and helped to complete a large and innovative project and this has delivered significant advances in our knowledge and understanding of drivers contributing to mortality in export cattle.

During the course of the project we encountered problems in accessing data and information relevant to the project objectives. The major reasons for this were that the export supply chain is complex and has multiple different providers performing services at steps along the way and there is no centralised data repository where data and information may be sourced. In some cases, exporters and AAVs assumed that project team members were collecting data from another source. In some cases, concerns over privacy and commercial sensitivities meant that individuals were reluctant to provide information. In the early stages of the project when there were relatively few eligible voyages, there were industry concerns over the ability of stakeholders to be able to recognise which ships or exporters might have been responsible for particular voyages when preliminary results were being released. Over time as more voyages were added to the project and results were aggregated, these concerns were ameliorated. The fragmented nature of data flows within the export supply chain provided a substantial challenge for the project team. There were datasets that we had hoped to collect and that we were not able to obtain because of these issues. For example, we were unable to obtain copies of daily voyage reports for enrolled voyages and this meant we did not get detailed data on daily mortalities and daily conditions on board the ship during an export voyage (including deck measures of temperature and humidity). We were not able to obtain details of all treatments and protocol testing results for cattle being sourced and prepared for export or during the voyage. This meant we were unable to assess the effects of specific treatments or vaccines that may have been administered to cattle before export or treatments administered during export. These issues did limit our ability to explore particular associations of interest, however they did not prevent the project from achieving all of the objectives.

The two areas where we did not achieve everything we had intended to, were in relation to collection of data to describe morbidity (part of Objective 3) and conducting on-farm investigations in conjunction with assembly feedlot investigations to increase our understanding of disease epidemiology (part of Objective 13).

We developed sick animal data collection sheets and dead animal / necropsy forms as part of our systems and procedures to facilitate collection of information on morbidity and mortality. We also had hoped to obtain copies of daily health reports and end of voyage reports for all enrolled voyages, which would have served as a source of additional information on morbidities. We were not able to obtain copies of daily or end of voyage reports from the Government, because of privacy restrictions, and exporters appeared to be reluctant to provide copies of these reports because of commercial sensitivities. A critical part of the embedded data collection systems was the requirement to limit systems to those that could be effectively embedded into routine operations conducted by AAVs while they were going about their regular day-to-day activities. Following our experiences with the pilot voyages and early stage 3 voyages, we chose to focus completely on mortality data because

of concerns from AAVs and exporters about AAVs spending too much time collecting information for the research study while they were employed on routine export voyages with a full set of commercial and legislative responsibilities. It was not possible for AAVs to routinely collect sick animal information in addition to their other tasks. If this project were being undertaken now, we would make every effort to design project data collection forms to meet regulatory reporting needs (daily and end of voyage reports) and lever morbidity and mortality data where possible off the back of these necessary compliance tasks. We tried to do this in the W.LIV.0252 project for morbidity information, but were not able to achieve this effectively.

The intent for Objective 13 was to explore potential risk factors that may be operating at either assembly depot level or at property of origin level. In designing this objective it was always recognised that on-farm activities would be constrained by cost and practicality. It simply was not possible to plan for on-farm visits and to travel to sufficient farms at a time when animals were being handled and might be available for examination and/or sampling. Constraints were associated with time, labour, access and cost. As a result, we focused on collecting samples from animals at assembly depots.

On both these occasions we made decisions based on protecting priority data collection in selected areas, that unfortunately had the effect of omitting some data collection from other desired areas. The overall impact of these issues on the broader success of the project was relatively small.

The two objectives where project requirements were met without requiring all of the proposed objective to be completed were Objectives 4 and 5. At the time the project started, we were proposing an open dialogue with industry to seek options for how research data might best be collected from systems that were embedded into routine day-to-day operations. We hoped that research activities would principally involve AAVs, but acknowledged that activities might involve both AAVs and stockpersons. If both groups were involved in research activities it would be very important to clarify responsibilities separately and in a complementary manner for AAV and stockperson to avoid confusion and redundant or inefficient actions. As discussions progressed it became apparent that AAVs would be the primary focus for research activities and therefore Objectives 4 and 5 were focused very clearly on AAV responsibilities.

With the benefit of hindsight, we would add substantially more resources in the design phase for communication, industry liaison, fine-tuning systems to embed them more directly into routine operations and for technical support of laboratory procedures. Having additional resources directed to these activities would allow the project team to complete key pathology testing in a more efficient manner and generate interim reports for results for communication to AAVs and exporters. Additional communication resource would also allow more effort to be directed at general communication with stakeholders including additional investment in newsletters, web-based communication and emails or phone calls, as well as face-to-face visits with co-operating exporters. It is possible that additional timely feedback and communication may have helped to maintain commitment to the project over time. This information should be considered by researchers in future considering some form of similar joint or collaborative approach to industry research.

In summary this project was large, complex, innovative and successful. It has laid the foundation for future field research driven by industry for industry benefit. It has generated substantial improvement in our understanding of cattle mortality risk on long haul voyages and particularly mortality due to respiratory disease. It has also contributed substantially to improved resources and systems for monitoring and reporting animal health and welfare outcomes.

13 Conclusions and recommendations

13.1 Conclusions

The main conclusions from this large project include:

- This project was based on large-scale collaboration between industry and researchers to embed research data collection into routine industry management of animal health. The approach allowed efficient, large scale investigation of relatively low probability events.

- Systems were developed and implemented with associated training and resource material to standardise and improve understanding of common diseases and management of health and welfare in export livestock. These systems underpinned the data collection methods employed in this project and offer sustainable improvements in industry management of animal health.

- Mortality percentages and rates and causes of death have been described for cattle exported from Australia to destinations including the Middle East, North Africa, Turkey and Russia.

- The project systems were applied to 20 voyages. There was a single voyage with a reportable mortality level and the overall percentage mortality across all 20 voyages was 0.38%. The major cause of mortality in enrolled voyages was respiratory disease (50% of all deaths that were studied) followed by musculoskeletal and injury conditions (15% of deaths).

- For the first time, advanced molecular diagnostic techniques have been applied to samples collected at pre-export feedlots and during the voyage to identify respiratory disease pathogens in healthy and diseased cattle and understand their role in the epidemiology of BRD in export cattle.

- For the first time, detailed descriptions of mortality rate (deaths per 1,000 cattle days) were reported for export voyages and patterns of daily mortality rate were described, showing the dynamic nature of mortality risk over the duration of an export voyage.

- Detailed pathology investigations, molecular testing for pathogens and patterns of mortality all supported the hypothesis that the epidemiology of BRD in export cattle is likely to be very similar to the epidemiology of BRD in land-based feedlot cattle.

- Suggestions have been made for follow up work to develop, implement and monitor risk mitigation strategies aimed at reducing BRD risk during export.

- The success of this project offers lessons for future projects that can be implemented for industry benefit. Suggestions have been made for additional projects that build on the lessons learnt from this project.

13.2 Recommendations

1. ***Consider development of a strategic approach to online performance data monitoring systems that are owned by industry and that incorporate measures currently being collected and reported on through the Shipboard Mortality Database (SMDB) and possibly through voyage reporting.***

   The concept is based around development of industry monitoring systems that can be implemented as part of routine export operations, that build on existing and well supported reporting systems (such as the SMDB), that provide performance reports of value to industry and that may contribute to industry QA and benchmarking.

   Cost-effective systems can be built and tested as a prototype incorporated into a research project (recommendations 3, 5 and 7).

   Development of hand-held systems for data collection could be tested as part of this approach and if successful, these can be applied in various parts of the supply chain (voyage, post-discharge, etc).

   Over time and in alignment with a broader strategy the concept could be extended to provide comprehensive coverage across the supply chain from property of origin in Australia to overseas port of disembarkation (ASEL) and for monitoring Australian-origin livestock in a foreign country (ESCAS).

2. ***Consider a follow up project that develops and applies BRD risk mitigation measures including vaccination, if appropriate, and implements these in a controlled manner to reduce BRD morbidity/mortality.***

   BRD was clearly identified as the major cause of death in long haul cattle voyages.

   Strategies should be developed from the findings of this report and in discussion with stakeholders from the land-based feedlot industry and researchers involved in recent BRD work in that industry. Selected strategies should be designed to be feasible under routine industry constraints and concurrent measures should be implemented to monitor performance to ensure they are cost effective.

3. ***Consider further investigation into the role of bovine coronavirus (BCoV) in BRD in feedlot and/or export cattle.***

   Our study and another contemporaneous study in eastern Australia were the first to detect BCoV in association with BRD in Australian cattle. Further work is required to characterise the genetic sequence of field isolates and understand the epidemiology of respiratory disease, as distinct from other diseases associated with BCoV. Given the possible similarities between land-based feedlot and live export supply chains with respect to bovine respiratory disease and the logistic difficulties in accessing and sampling animals during export, it may be more effective to do detailed investigations on pathogenesis and epidemiology of BCoV in land-based feedlot cattle and then undertake simpler studies in export cattle to test broader hypotheses to determine if the same conclusions may be reached about the disease in export cattle.

4. ***Consider a follow up project that develops and applies strategies to mitigate risks for musculoskeletal conditions during voyages.***

   Musculoskeletal injuries and associated conditions including infections were the second most important cause of death in long haul voyages. These conditions are noteworthy because the initial injury may be relatively minor and might be of relatively little consequence for animals on pasture, but are difficult to treat successfully and may lead to progressive deterioration and death on board a ship.

   Strategies need to be focused on prevention through measures such as facilities design and construction, animal selection and preparation and through low-stress handling. There may also be opportunities to improve the ability to treat affected animals during a voyage to improve recovery rates.

   Strategies could be implemented and monitored at low marginal cost, if an industry monitoring system was in place.

5. ***Use the findings from this project to contribute to an update of the Veterinary handbook for the live export industry.***

   The current project (and related projects accompanying this one) developed a range of resource and training material for industry benefit. This material needs to be updated, where appropriate, and reviewed to ensure it continues to be current and of value to industry.

   The current project has described causes of death in export cattle. The findings can be used to refine methods for diagnosing causes of death on board export ships using ante-mortem observations and gross necropsy alone. These developments will improve the quality of reporting from voyages, understanding of contribution of specific diseases to morbidity and mortality over time and contribute to a higher standard of health care and welfare outcomes.

   A range of digital images were collected in the course of the current project. Updating the handbook is expected to incorporate a digital library of images to aid in diagnosing and managing conditions in export animals.

   Other material from the project specific handbook should be considered for inclusion in the updated handbook, if appropriate (knife sharpening, standardised list of equipment for conducting necropsies on ship, how to take and store digital images).

6. ***Consider applying the lessons and approaches developed in this project to the sheep export supply chain to monitor impact of strategies aimed at reducing losses from salmonellosis and inanition.***

14 Acknowledgements

This project could not have occurred without the dedication and commitment from co-operating exporters and their staff, and the Australian government accredited veterinarians (AAVs) who willingly attended project meetings, training sessions and acted as data collectors during export voyages.

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Drs Mandy O'Hara and Ann Barnes from Murdoch University, Dr John Creeper and Mr Greg Norman and Dr Mark O'Dea from the Department of Agriculture and Food, Western Australia all played pivotal roles in performing laboratory testing of tissues and other samples collected during this project. Dr Tristan Jubb from Livestock Health Systems Australia provided valuable input into the design of the project and development of systems and procedures and associated training and resource material. Dr Michael McCarthy provided valuable input as an AAV with experience in routine commercial voyages and combining R&D with regular export activities. Dr Ben Madin provided expertise in spatial data analysis for and web-based systems. Ms Tracey Locke and Mr Russell Hunter assisted in project management and report preparation.

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15 Communications

Moore SJ. Investigating causes of mortality in live export cattle. This thesis has been presented for the degree of Doctor of Philosophy at Murdoch University, WA, May 2014.

Moore, S.J., O'Dea, M.A., Perkins, N., Barnes, A., O'Hara, A.J. Mortality of live export cattle on long-haul voyages: pathological changes and pathogens. *Journal of Veterinary Diagnostic Investigation*, 2014 26(2):252-65 (Chapter 4).

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Moore, S.J., O'Dea, M.A., Creeper, J., Perkins, N., Barnes, A., O'Hara, A.J. Causes of mortality in live export cattle on long haul voyages. American College of Veterinary Pathologists Annual Meeting, November 16th – 20th 2013, Montreal, Canada. Poster presentation.

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15.1 Public media

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Respiratory disease causing export cattle deaths. Interview and article by Carmen Brown for ABC Radio, 11th March 2014. Available at: http://www.abc.net.au/news/2014-03-11/export-cattle-deaths-linked-to-respiratory-disease/5310412.

16 Capacity building

This project formed the substantive basis of Dr Jo Moore's PhD training through Murdoch University, Western Australia.

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