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Investigating the relationship between Salmonella-inanition and property of origin

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Abstract

This report provides a review of current knowledge concerning salmonellosis and inanition as causes of mortality in live export sheep and the potential associations between mortality and farm-level risk factors. Difficulties in investigating farm-level risk factors for mortality in export sheep are described. The report then identifies several activities aimed at developing capacity to monitor, investigate and improve health and welfare outcomes in export sheep. These include the development of an integrated industry approach to ongoing monitoring and surveillance of mortality (and morbidity), methods to investigate inappetence and behaviour in export sheep, vaccination of sheep against Salmonella and the use of a risk framework to identify interventions to minimise risk of inanition and salmonellosis. These approaches incorporate the capacity to monitor and investigate risk factors for salmonella and inanition regardless of the level of the export structure at which they may operate.

Executive summary

This report aims to provide a review of knowledge concerning factors that influence mortality in live sheep with a particular focus on salmonellosis and inanition and those risk factors that may be operating at the farm level. The second part of the objective is to produce robust and scientifically sound proposal(s) that investigate potential risk factors.

The literature review component of this report makes particular use of two recent reports on inanition and inappetence (Barnes et al 2008) and on salmonellosis and other causes of mortality in export sheep (Makin et al 2008) as well as other earlier literature and reports. Difficulties in investigating farm-level risk factors for mortality in export sheep are described. The report then identifies several activities aimed at developing capacity to monitor, investigate and improve health and welfare outcomes in export sheep. These approaches incorporate the capacity to monitor and investigate risk factors for salmonella and inanition regardless of the level of the export structure at which they may operate.

Development of an integrated monitoring and surveillance capacity
The live export industry is heavily regulated and has a number of monitoring and reporting obligations concerning animal health and welfare. The authors believe there is a great deal of potential to adapt routine industry practices to facilitate industry management of regular shipments (make things work more smoothly and effectively for industry operators), and collect better information for industry purposes including reporting to AQIS and contributing to effective quality assurance and research.

An example of this approach has been seen in the development of the prototype information management system as part of B.LIV.0123 activities (Makin et al 2008). It is intended that the same broad approach will be used for W.LIV.0252 which started in June 2009.

A combination of a sustainable, broad monitoring capacity (scanning surveillance in a biosecurity sense) and targeted, more detailed studies to investigate particular problems or test specific hypotheses is considered to offer the best mix of capacity when adverse outcomes are uncommon and unpredictable. This is the mix of attributes that is considered to be robust and scientifically sound in investigating property of origin effects associated with mortality in export sheep.

It is recommended that the Objectives outlined in this report be used as the basis for research project(s) aimed at development of an information management system and associated standardised recording systems and procedures for the export industry. This application should build on the experiences of Makin et al (2008) and the proposed activities being initiated in the cattle export industry through W.LIV.0252. It is anticipated that this monitoring capacity would provide ongoing descriptions of mortality risk and provide limited information on risk factors. This information is then likely to serve as an important foundation that will inform and underpin subsequent studies that may investigate specific issues in more detail. As an example crude mortality data at the consignment or vendor property level should allow identification of simple associations between factors such as month or season, property or geographic area and mortality risk, and particularly of associations that are repeated over time. This information can then be used to inform more detailed studies that may involve additional activities to investigate these associations. While the general approach is not different to what has been tried in the past, the ability to have routine on-going monitoring across the export industry to assess crude associations over time, represents an important advance in the approach to investigation of outcomes that appear to be rare and associated with complex and variable causal webs.

Experimental studies to investigate inappetence and inanition
A recent application in this area is believed to have been approved for funding[a]. The application describes a relatively large-scale investment in the capability to electronically track large numbers of animals under experimental conditions using radio tracking and purpose designed ear tags. The methods offer potential to record relatively simple information about feed trough access and in some cases may allow recording of detailed spatial and positional data on individual animals within a mapped area, such as a feedlot shed.

While it has a relatively large up-front cost, the establishment of this methodology is certain to represent a long term investment with many years of subsequent experiments being able to be done at reduced cost. The methods have the potential to facilitate a great deal of research into appetite control and inappetence as well as other areas of interest for the export industry associated with animal behaviour, welfare and health.

This report does not propose further action at this stage given that the research application remains under consideration at the time this report was prepared.

Investigation of farm-level factors associated with mortality risk
The use of field observational studies to investigate farm-level risk factors that may be associated with risk of mortality is discussed at some length in this report. These approaches are likely to be successful only if:

- they involve reliable tracing of animals to line and to farm of origin (property that the sheep were on immediately before they were transported to the assembly feedlot)
- they involve collection of data from large numbers of lines of sheep over a long time period
- farm-level risk factors are truly responsible for variation in mortality that appears to exist at the line-level and
- data/information is collected on the right risk factors to facilitate detection

Because of clustering of sheep in lines there is considered to be benefit in a multi-stage approach to sampling with a large number of lines being enrolled and a sample of animals being followed from each line. A sampling strategy is outlined in Appendix 1 and involves following a sample of sheep from all lines of exported sheep over a two to three year period. The combination of low background mortalities and variable and unpredictable occurrence of mortality events means that the approach would be risky and not robust. For these reasons, this approach is not favoured by the authors.

An alternative approach which is favoured by the authors is to develop the two component capabilities as described in the previous two sections.

The combination of these two approaches provide capacity to describe and investigate risk factors for the two major causes of mortality (inanition and salmonellosis) no matter where those risk factors may be operating (animal, farm, truck, feedlot).

Development of more detailed terms of reference for studies that may be conducted under this section is completely dependent on results from analyses of data collected from a functioning IMS as described under section 8.1 and to a lesser extent on an existing capacity to perform experimental studies under controlled conditions as described under section 8.2. In the absence of such information it is not possible to present detailed methods and therefore this section is not developed further in this report.

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[a] Backgrounding and feedlotting strategies to address Inanition in the Livestock Export Industry. Application by Anne Barnes et al (2008).

Understanding and managing Salmonella exposure

Very few sheep are shedding Salmonella organisms when they enter an assembly depot. Even when most sheep are not shedding on arrival, over time in a system designed to accommodate heavy throughput of animals, the assembly feedlot will eventually be contaminated with Salmonella through organisms shed by clinically infected animals and passive shedders. It is generally expected that there will be a low prevalence of Salmonella infections in sheep when export demand is low and assembly yards are either stocked at lower rates or are rested between shipments, and higher prevalence of Salmonella infections under conditions of higher throughput. A variety of factors then influence this overall pattern and may increase or reduce the risk of salmonellosis including factors associated with farm-of-origin, transport, feedlot and extrinsic factors such as climate.

Project objectives are described that contribute to the development of assays to detect and measure severity of Salmonella exposure risk in assembly feedlots (and other areas such as farm of origin and export ship) and subsequently use these to both describe exposure risk and assess interventions intended to reduce exposure risk in export sheep.

Vaccination against Salmonella

A recently completed report (Perkins et al 2009) has provided a cost-benefit analysis on the feasibility of oral vaccination against salmonellosis in export sheep. The report indicates that development of an oral vaccination against Salmonella appears to be cost effective and that it is worthy of further research. It is noted that the marginal return per sheep is positive but very low and is influenced by assumptions concerning exchange rate, sale price, vaccine efficacy and incidence of mortality due to Salmonella. There are also intangible benefits in preventing mortality spikes associated with Salmonella that favour further investigation of the vaccine.

It is recommended that industry consider the report (Perkins et al 2009) with a view to further investing in research leading to the development and trialling of an orally administered vaccine against salmonellosis and if results are favourable, to work towards registration of a vaccine in Australia for use in sheep intended for export.

Contents

1 Introduction

This document provides a synopsis of current knowledge of the relationships that exist between inappetence, salmonellosis and inanition in the live sheep trade including presentation of causal factors contributing to both salmonellosis and inanition in a unified causal web. Previous studies have reported line-level variation in incidence of mortality that is suggestive of risk factors that may be operating at the farm level. These findings are discussed and difficulties in identification of farm-level risk factors are identified. The report then outlines a number of activities intended to develop capacity to monitor and investigate mortalities in export sheep including investigation of risk factors at any level in the structural hierarchy of the export industry.

2 Objectives

Review and development of a methodology and budget incorporating:

- Review of existing literature to document current knowledge of the causes of mortality in sheep during livestock export that are influenced by on-farm factors, with a focus on Salmonella and inanition.
- Develop a scientifically sound methodology and robust implementation plan to address the current gaps in knowledge regarding the reasons for variation in the number of mortalities between farm groups.

3 Inappetence and inanition

There are a number of terms used in relation to reduced appetite in animals that may not be interpreted in a consistent manner and it is appropriate to define these at the beginning of any discussion on this topic.

- Inappetence: reduction in or lack of appetite with consequent reduced food intake (Blood and Studdert 1999)
- More (2002a) described **persistent inappetence** as the complete and voluntary refusal to eat
- Anorexia: complete absence of appetite and refusal of food (Radostits et al 2000)
- Inanition: state of exhaustion resulting from lack of food and waterb. The term is commonly considered to be an end-stage condition and has been used to describe deaths in export sheep attributed to prolonged reduction or cessation of feed intake (Richards et al 1989).
- Starvation: prolonged deprivation of food and consequent effects on the body (Anderson 2007)

It is noted that there is variation in interpretation applied to these terms in the scientific literature. For example Radostits et al (2000b) describe starvation as the complete deprivation of food with rapid depletion of glycogen stores and a change over in metabolism to catabolism of fat and protein. The same text then describes inanition as malnutrition or incomplete starvation where the diet is insufficient in quantity but contains essential nutritional components and is compatible with life. Inanition is then linked to the same physiologic and metabolic changes as described for starvation but to a lesser degree of severity (Radostits et al 2000b).

Inappetence in ruminants may be influenced by a large number of intrinsic and extrinsic factors (Barnes et al 2008), including some or all of the following: age, source of sheep, breed, sex,

[b] Merriam-Websters online dictionary, http://www.merriam-webster.com

fatness, season, climate, feed (type of feed, method of delivery, trough space and placement), and stressors (farm, transport, mixing, stocking density, concurrent disease). More detailed information on appetite control and causal factors that may contribute to inappetence is presented in the review by Barnes et al (2008).

If insufficient energy is consumed to meet the animal's needs then this constitutes a negative energy balance, and results in mobilisation of additional energy from body reserves with increased risk of ketosis and inanition. Body fat is metabolised into free fatty acids or NEFAs which can be stored in the liver or converted by the liver to either glucose or ketones such as beta-hydroxybutyrate (BOHB). Excessive production of NEFA and ketones may have adverse effects including liver dysfunction associated with excessive fatty acid deposition (fatty liver) and ketoacidosis. These changes can themselves result in altered mentation and further depression of appetite in an already inappetent animal (Radostits et al. 2000).

Not all animals in negative energy balance will become ketotic and there is evidence to suggest that sheep are generally resistant to energy deprivation with the exception of ewes in late gestation (pregnancy toxaemia) where the overwhelming energy demands of multiple foetuses cannot be met by dietary intake. Moloney and Moore (1994) found that prolonged (8 weeks) restriction of energy (feeding 25% of that required for maintenance) did not result in sustained elevation in blood BOHB or clinical ketosis. Earlier studies have also noted that ketosis in non-pregnant sheep is uncommon and requires extended periods of inanition (Shaw and Daugherty 1946).

Figure 1 presents a diagrammatic representation of factors influencing energy balance and inanition.

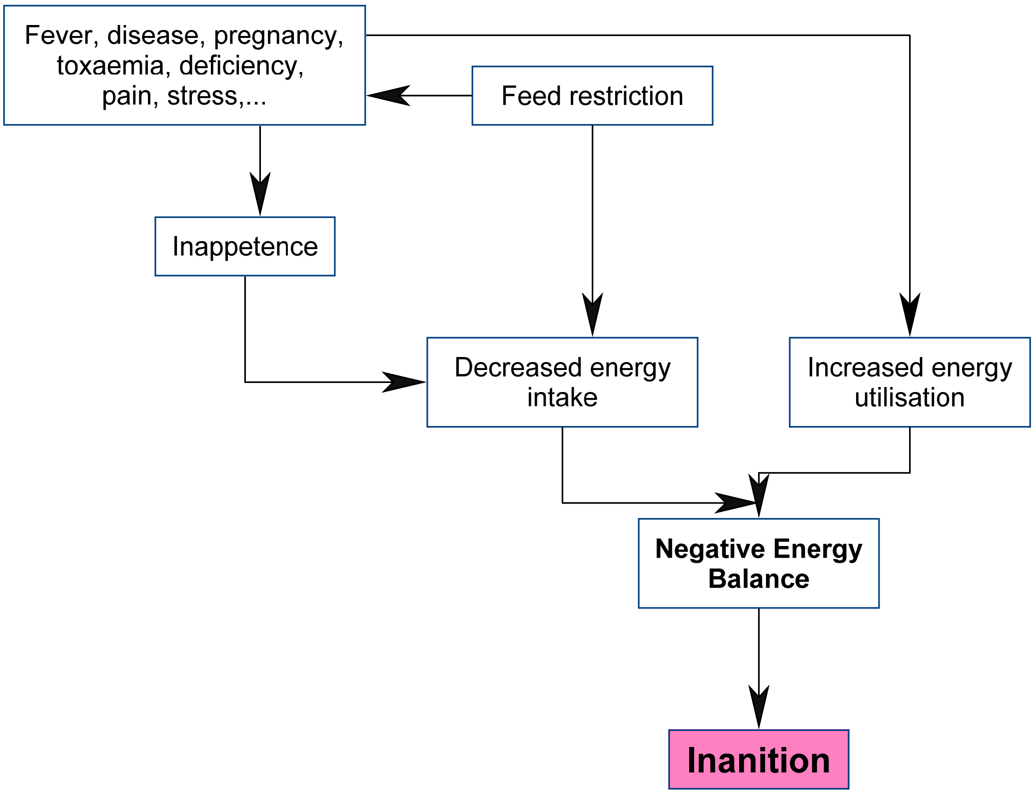


Figure 1: Causal pathway for factors influencing energy balance and inanition (Adapted from Makin 2009)

There is evidence to suggest that fatness (higher average condition score) is associated with increased risk of non-feeders in export sheep and with shipboard mortality (Higgs et al 1991) and it is hypothesised that fatter animals coming off better quality feed may be less tolerant of negative energy balance and more likely to develop ketosis in response to negative energy balance (Higgs et al 1991; Richards et al 1991).

Sheep that are persistently inappetent are at risk of progressing to a state of inanition and may then die due to lack of energy. Higgs et.al (1991) reported that 85% of inanition deaths on ship were considered to be a direct consequence of persistent inappetence that began in the assembly depot. It is important to note the time scale normally associated with a progression from inappetence to inanition and subsequent death. In normal healthy sheep that become persistently inappetent it is suggested that it would take two to four weeks for animals to die from inanition (J House, Personal communication). In cases that are complicated by conditions such as reduced access to water, occurrence of ketosis, lack of resistance to inappetence (older, fat wethers coming off high quality pasture), or that suffer secondary or other concurrent conditions in association with or addition to inanition, this time frame may be shortened. The expected impact of ruminant resistance to reduced feed intake and the time required for an animal to die from inanition alone is that death from inanition alone may be more likely to occur during the voyage and less likely to occur during the assembly feedlot unless additional factors or conditions were involved.

In the Australian sheep export industry, higher proportions of inappetent sheep and higher mortality rates have been identified in sheep with greater fat reserves (Richards et al 1989, 1991; Higgs et al 1991), in mature sheep (Norris et al 1989ab), in sheep from areas with a long (greater than 7 months) pasture growing season (Higgs et al 1999) and in sheep exported during the second half of the year (Norris and Norman 2004). Higgs et al (1991) found that in lines of sheep with a high condition score there was a higher proportion of assembly depot non-feeders and higher mortality on ship, with fat sheep (condition score 3 or greater) being almost twice as likely to die on ship when compared with sheep that were not fat.

It has been proposed that the influences of season, age and adiposity on feeding behaviour and subsequent mortality may be influenced by naturally occurring seasonal-cycles that affect sheep (Higgs et al 1991; Richards et al 1991). Richards et al (1991) measured biochemical parameters in blood from feeders and non-feeders at two times of the year (May and August). Non-feeders had elevations in non-esterified fatty acids (NEFAs) that were consistent with negative energy balance. Non-feeders in August tended to have a shorter and less marked rise in NEFAs and a more pronounced rise in plasma urea concentrations. The authors hypothesised that lower pasture availability in Western Australian conditions in the first half of the year were likely to be associated with a strong appetite drive in sheep. During this period, sheep may often be in a state of reduced energy intake and even negative energy balance and are metabolically accustomed to utilising body fat stores. Sheep at this time of year were considered to be able to cope well with prolonged inappetence or feed deprivation. Conversely, in the second half of the year, when pasture availability is generally high, sheep were likely to have been in a positive energy balance for some time and are generally laying down fat stores. Richards et al (1991) concluded that under conditions of high availability of quality pasture, sheep were less able to adapt to prolonged inappetence or feed deprivation by metabolising body fat and instead will start to mobilise amino acids from muscle to meet energy needs.

Most sheep that are non-feeders in the assembly depot have been shown to start feeding during the early part of the voyage (Norris et al 1989b, Norris et al 1990). However, inappetence in the assembly depot has been proposed as a critical risk factor for death during shipping with two thirds of all deaths being linked to failure of sheep to eat (Richards et al 1989, 1991). In three separate studies, assembly depot non-feeders have been shown to be 3.2 (Norris et al 1989b), 6.83 (Higgs et al 1996) and 13.9 (Norris 1990) times more likely to suffer mortality on ship than sheep that ate during the assembly period. Factors other than inappetence also influence mortality risk since some lines of sheep experience elevated mortality without evidence of inappetence.

The percentage of non-feeders per line of sheep during lot-feeding ranged from 0 to 59.1%, with a majority of assembly depot non-feeders originating from a limited number of farm groups, indicating that farm-level factors may be influencing risk of inappetence (Norris et al 1989a). Denominators for each line were not reported so actual numbers of affected sheep and counts of totals (sheep at risk) are not available. It is noteworthy that one of five voyages (voyage 2) appeared to be particularly affected with non-feeders during lot-feeding and this was attributed by the authors to the fact that these sheep were provided with hay throughout the lot-feeding period allowing animals the choice of eating hay or eating pellets. In the remaining four voyages, hay was either not fed at all (n=3) or was fed for only the first 6 days of the 11-day lot-feeding period (n=1). When data from voyage 2 were separated from other voyages the results were less dramatic. In voyage 2 the percentage of non-feeders per line ranged from 1.9 to 59.1% and the highest median percentage of feeders per line was 68%. In the other four voyages, the percentage of non-feeders per line ranged from 0 to 19.1% and the highest median percentage of feeders per line was 99.9% (Norris et al 1989a). It seems highly likely that estimates of non-feeders in voyage 2 were artificially elevated since they were based on whether or not sheep were marked as they approached the feed troughs containing pellets. Since hay was available throughout the lot-feeding period in this particular voyage, sheep could have been eating hay and ignoring pellets and be classified as non-feeders.

Norris et al (1989b) investigated on-farm management factors that may contribute to mortality, including purchase history, prior experience with transport, yarding, supplementary feeding, time of shearing and social interactions. No consistent associations were identified between on farm management and mortality during export so the effect of these factors on outcomes in the live sheep trade remains unclear. The same study also investigated transport related factors (distance and time on truck from property to assembly depot, and time off feed) and reported no association between transport factors and mortality risk (Norris et al 1989b). There were associations reported between observations made while sheep were in the assembly feedlot and with subsequent voyage mortality. Inappetence (failure to eat pellets) in the feedlot was associated with increased risk of shipboard mortality. Associations between inappetence in the feedlot and specific causes of death during the voyage were not consistent. In some voyages there was an association between inappetence and shipboard deaths due to salmonellosis and in other voyages with salmonellosis and inanition combined. In one voyage with elevated deaths due to salmonellosis there was no association with earlier feedlot inappetence. There was no consistent association between good feeders in the late feedlot period and low mortality risk during the voyage. There was also no association between the type of feedlot housing (sheep fed in sheds vs outdoors in paddocks) and voyage mortality. The authors noted the possibility of low statistical power due to practical constraints on sample size (sheep in a line and number of lines in the study) as a result of the intensive nature of the study and the requirement to post mortem every dead animal.

4 Salmonellosis

Salmonellosis refers to the clinical disease associated with Salmonella infection. The common clinical signs of salmonellosis in sheep include anorexia, fever, diarrhoea, depressed mentation and death.

Several factors are important in the development of salmonellosis. These include Salmonella challenge (number of bacteria and virulence of the strain), specific immunity to Salmonella infection (influenced by previous exposure to Salmonella) as well as general state of health and resistance to infection (influenced by innate immune response capacity, concurrent disease, nutrition and stress). Ingestion is thought to be the most common route of infection though exposure may also occur via inhalation or conjunctival contamination.

The prevalence of sheep shedding Salmonella on entry to export assembly depots has been shown to be very low (<1%; Higgs et al 1993). This is consistent with findings reported by Makin et al (2008) that the proportion of positive salmonella cultures in faecal and environmental samples consistently increased from early in the assembly period to late indicating that salmonella contamination and sheep exposure increases during the assembly period. The high throughput of sheep in a busy assembly feedlot means that over time there is a high likelihood of Salmonella organisms being shed into the environment even though the individual animal prevalence in arriving sheep is very low. Clinically infected animals and passive shedders are thought to be the major potential sources of Salmonella organisms for exposing other susceptible animals either directly or indirectly through contamination of water, feed and the environment. Salmonella organisms can survive for months to years in the environment and under favourable environmental conditions Salmonella are capable of proliferation in the environment to increase the level of environmental contamination (Murray 2000).

Export assembly depot receival and load-out yards are frequently contaminated with Salmonella and the level of environmental contamination is considered to rise during the assembly phase prior to load out of an assembly feedlot (Kelly 1996). Under periods of high demand, assembly depots have a heavy use pattern with new animals arriving very soon after a previous shipment has been loaded out. This provides an opportunity for Salmonella loads in the yard environment to build in a cumulative fashion over time. The expected pattern of disease associated with Salmonella infection in association with sheep that are exposed to infection after arrival at the feedlot is low incidence of disease initially with a rise in incidence during the latter phase of the assembly period and on occasion continuing into the voyage component of the process.

The outcome of the host-pathogen interaction at the individual animal level is variable and ranges from resistance to infection to acute, fulminant bacteraemia, endotoxaemia, and death. There are a large number of causal factors that may influence the outcome of host-pathogen interactions and many of these factors are likely to be capable of rapid and frequent change both within and between animals and lines. Variable host immunity may reflect differences in previous Salmonella exposure and presence/severity of stressors including periods of feed deprivation prior to arrival. Variability also occurs in relation to the magnitude of the Salmonella challenge and the virulence of Salmonella in the assembly depots. This variability reflects the influence of sheep throughput and of environmental conditions on Salmonella proliferation, virulence, and persistence in the environment.

Healthy sheep have sufficient host resistance to be reasonably resistant to exposure to Salmonella. However, stressors such as transport, yarding, inappetence, concurrent disease and inclement weather can all decrease host resistance and increase susceptibility to Salmonella. While healthy sheep with no apparent predisposing conditions can still be infected with Salmonella and develop clinical disease, there appears to be a vast difference in susceptibility. Exposure doses as low as hundreds of Salmonella organisms may be sufficient to cause disease in compromised sheep while as many as 10^9 organisms may be required to cause disease and mortality in healthy sheep (Wray and Linklater 2000).

The growth of Salmonella in the rumen following ingestion is also influenced by dietary intake and gut factors in a relatively complex interplay of causal factors.

The growth of Salmonella in the rumen is normally inhibited by high concentrations of volatile fatty acids and a low rumen pH (normal rumen pH is 5.5-6.5; Chambers and Lysons 1979; Mattila et al 1988). When sheep fail to eat, production of volatile fatty acids is reduced and rumen pH in anorexic sheep therefore rises and may approach 7 or 7.5. Reduced feed intake either through interruption of feeding for one or more days (during travel for example), or through anorexia may therefore result in an increase in numbers of Salmonella in the rumen. In contrast, Salmonella disappear rapidly from the rumen of regularly fed ruminants (Brownlie et al (1967). In addition to inhibiting bacterial replication, volatile fatty acids can also reduce Salmonella virulence (Boyen et al 2008).

The biochemical explanation for these effects is complex and some of the effects are actually dependent on the type of fatty acids produced. For example, butyrate and propionate cause suppression of Salmonella invasion of epithelial cells in vitro, while acetate does not suppress cell invasion. Lawhon et al (2002) explained this effect on the basis of changes in Salmonella pathogenicity island (SPI-1) expression. It has been found that SPI-1 contains the Salmonella virulence genes required to invade epithelial host cells during early stages of infection. These genes are transcriptionally regulated by the HilA protein, also encoded by a gene of the SPI-1 pathogenic island (Durant et al 2000). Butyrate reduces HilA and some of the genes under its control (Fernandez-Rubio et al (2009). Inhibition of HilA expression by butyric acid is pH dependent, and at pH 7 there is nearly no influence on HilA expression (Papezova et al 2007). Inhibition is favoured by a more acidic pH. Reduction in feed intake and the consequent rise in rumen pH is therefore associated with loss of inhibition of these regulators, creating an environment that favours rapid proliferation of Salmonella.

Feeding after a period of starvation is also associated with multiplication of Salmonella (Frost et al 1988; Grau et al 1968). Dietary changes that result in clinical or subclinical ruminal acidosis may increase the risk of salmonellosis because rumen acidosis results in disruption of normal fermentation and the subsequent production of lactate (Chambers and Lysons 1979). Lactate is a stronger acid than the other volatile fatty acids (acetate, proprionate, and butyrate), therefore it is more dissociated than the weaker acids at an equivalent pH. Volatile fatty acids only diffuse across the bacterial cell membrane in the un-dissociated form (Warnecke and Gill 2005), and then dissociate within the bacterial cell. In its dissociated form lactate is unable to diffuse across the bacterial cell membrane and therefore any beneficial impacts of intra-cellular fatty acids are lost. The lactate acidosis also favours the less fastidious Salmonella in contrast to other rumen micro-organisms and Salmonella can then multiply rapidly using the available substrate (Chambers and Lysons 1979). In addition, ruminants with ruminal acidosis are often anorexic for variable periods reflecting systemic endotoxaemia and acidosis. Anorexic individuals recovering from ruminal acidosis may then incur a rise in rumen pH as a consequence of anorexia and the buffering affect of saliva which in ruminants is high in bicarbonate. These changes illustrate some of the complexities in understanding factors that influence Salmonella proliferation and virulence and attempt to explain how Salmonella can proliferate under conditions of elevated rumen pH and in cases where rumen pH may be reduced, with the effects mediated largely by the type of fatty acids that may be produced.

Faecal culture surveys performed during feedlot assembly of export sheep have documented an increase in Salmonella shedding during the assembly period, indicating that sheep become infected with Salmonella during the assembly period. The proportion of sheep shedding Salmonella prior to load out from the assembly depots ranges from 7 – 93% (Makin et al 2008;

Kelly 1996; Higgs et al 1993). The Salmonella serotypes isolated from assembly yards, those shed by sheep during the assembly period, and those isolated from the tissues of sheep that have died of salmonellosis are all similar (Makin et al 2008; Kelly 1996). When combined with the very low prevalence of Salmonella shedding (<1%) in sheep as they arrive at the assembly yards, these findings suggest that the assembly facility is the most likely source of Salmonella infection for many sheep that subsequently die of salmonellosis (Makin et al 2008; Kelly 1996). Salmonella shedding exponentially amplifies Salmonella contamination of the environment. Infected animals may excrete 10^8 to 10^{10} Salmonella per gram of feces (De Jong and Ekdahl 1965). As environmental Salmonella contamination increases, the balance between challenge dose and herd immunity is tipped in favour of the pathogen. High sheep throughput has previously been proposed as a risk factor for salmonellosis during the assembly period (Kelly 1996). As sheep are run through the same yards for receival and load out, high throughput will increase the Salmonella challenge encountered by sheep on arrival at assembly depots and when sheep are housed or handled in assembly paddocks or yards that have been used in previous assembly periods.

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

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

In summary, scientific knowledge of Salmonella epidemiology and pathogenesis is consistent with observations of disease in export sheep attributed to salmonellosis. Salmonella are ubiquitous but in healthy animals managed under reasonably extensive livestock systems such as those contributing sheep from property of origin to the export industry, the prevalence of Salmonella organisms in faeces is expected to be very low and may be due to latent or clinically inapparent infection or a very low prevalence of clinical disease.

In some cases there may be logical explanations for sheep having a higher prevalence of shedding at the time of arrival at an assembly feedlot including having higher rates of clinical disease. These situations may be expected in lines of sheep that have been subjected to conditions likely to involve elevated exposure to Salmonella and increased risk of infection including mixing of animals in saleyards or dealer operations, frequent or prolonged transport movements, reduced feed access, or other stressors. There are anecdotal reports of individual lines of sheep having elevated incidence of salmonellosis during the assembly feedlot or voyage periods, and being linked to one or more of these historical experiences prior to arriving at the assembly feedlot.

Even when the expected prevalence of Salmonella shedding is very low, the large numbers of sheep that are exported mean that there is a continual risk of introduction of sheep into the assembly feedlots that are shedding organisms. This leads to environmental contamination and exposure/infection of other sheep. Under suitable climatic conditions, Salmonella organisms can rapidly proliferate in the environment leading to heavier levels of contamination and greatly elevated risk of exposure and infection of other sheep. As more sheep get infected there is a large increase in the number of organisms being shed into the environment and more opportunity for direct and indirect exposure of other animals. This pattern is consistent with observations of low prevalence of Salmonella infections in sheep when export demand is low and assembly yards are either stocked at lower rates or are rested between shipments, and with increased risk of exposure and occasional spikes of salmonellosis that may occur under conditions of higher throughput. A variety of factors then influence this overall pattern and may increase or reduce the risk of salmonellosis including factors associated with farm-of-origin, transport, feedlot and extrinsic factors such as climate.

5 Relationships between inanition and salmonellosis

5.1 Case definitions

A number of studies have investigated mortalities in export sheep and have attributed deaths to various causes based on a combination of antemortem clinical signs, gross changes seen at post mortem, histologic examination of tissue samples collected at post mortem and microbial culture of samples collected either antemortem or post-mortem. A comparative understanding of results from different studies is helped by an appreciation of the criteria used to assign deaths to various causes.

Richards et al (1989, 1993) described the following diagnostic criteria in distinguishing inanition and salmonellosis:

- Inanition: less than 1.5% rumen solids and absence of other significant gross or histologic lesions (percentage rumen solids calculated as the weight of the solid fraction of the rumenoreticulum divided by the empty body weight – body weight of dead animal minus the combined total weight of the rumenoreticulum and abomasums).
- Animals with acute enteric lesions consistent with acute salmonellosis and evidence of inanition, were classified as primary inanition.
- Salmonellosis: gross and histologic lesions of enteritis and septicaemia
- Animals with evidence of chronic enteric lesions and inanition were classified as primary salmonellosis.
- Animals with salmonellosis were classified as septicaemic, acute, subacute, or chronic based on a combination of gross and histologic signs.

Makin et al (2008) classified conditions using a slightly different approach:

- Inanition: very low or absent rumen contents (reduced rumen solids), poor body condition and an absence of other significant pathology (i.e. gross enteritis or pneumonia). Affected animals may show gall bladder enlargement and evidence of fat mobilisation.
- Enteritis:
- Acute enteritis was characterised by inflammation (reddening, thickening) and congestion (engorgement with blood) of the abomasum, small and large intestine, as well as enlargement of the mesenteric lymph nodes. Affected animals may also show signs of septicaemia including marked congestion of the mesenteric vessels.
- Chronic enteritis was characterised by enlarged mesenteric lymph nodes and less severe changes in intestines, including typical ‘tiger stripe’ lesions in the caecum.
- Enteritis and inanition: Animals displaying some combination of gross signs of both of the above categories. Enteritis lesions may not be as severe and are often chronic. Rumen solids are usually low to moderate (rather than absent) and depletion of body stores is less severe.

5.2 Mortality investigations

Studies in the 1980s and early 1990s reported that salmonellosis was the most common cause of death in assembly feedlots (42.7% of assembly deaths), followed by miscellaneous diseases, trauma (23.8%), inanition (10.2%) and diseases associated with excessive feed intake (7.3%) (Richards et al 1989). During the voyage inanition was the most common primary cause of death (37.9% of voyage deaths) followed by salmonellosis (24.1%) and trauma (10.3%). In some voyages, salmonellosis was the most important cause of death, associated with outbreaks of subacute salmonellosis and septicaemia (Richards et al 1989). A subsequent paper by Richards et al (1991) reported that inanition was the most common cause of mortality in live sheep exports, accounting for 65% of all deaths.

These results were consistent with those of a Portland based mortality investigation in which inanition and salmonellosis were shown to account for 61-75% of mortalities (Kelly 1996).

Higgs et al (1993) reported that lesions and deaths due to salmonellosis were observed exclusively in inappetent sheep while other study animals that did feed (not inappetent) did not develop similar lesions. In these studies inappetent sheep were detected using marker bars located at feed troughs. In three separate studies, assembly depot non-feeders have been shown to be 3.2 (Norris et al. 1989b), 6.83 (Higgs et al. 1996) and 13.9 (Norris et al. 1990) times more likely to suffer mortality on ship than sheep that ate during the assembly period. In addition sheep that remain persistently inappetent during the ocean voyage are at even higher risk of dying and account for a majority of mortalities during the export process (Richards et al. 1989).

In the recent studies associated with B.LIV.0123 and reported by Makin et al (2008), marker bars were not used to detect inappetence and inappetence was diagnosed mainly based on elevations in serum NEFA or BOHB concentration or on post mortem changes. Very few deaths were observed in the assembly period during B.LIV.0123 and results on assembly-period mortality were based on a small number of assembly periods where project team members were present to perform post mortems and establish cause of death. Some lines of inappetent sheep were observed to have higher mortality while other lines of inappetent sheep did not have higher mortality when compared to lines that were feeding well. In some groups there appeared to be evidence of inappetence serving as a predisposing factor for subsequent Salmonella shedding and salmonellosis. In other groups, Salmonella infection appeared to occur in sheep with little evidence of prior inappetence and these sheep then showed elevated mortality due to salmonellosis followed in some cases by mortalities due to inanition that appeared to be secondary to salmonellosis. The overall mortality rate was low in assembly feedlot periods that were studied intensively and was considered to represent background mortality in the export system. The authors noted that in general post mortem examinations revealed little evidence of inanition. These findings appeared to differ from previous reports including those reporting up to 10% of assembly deaths due to inanition (Richards et al 1989) and reports that lesions and deaths due to salmonellosis were observed exclusively in inappetent sheep (Higgs et al 1993). While there were minor differences in the way case definitions were applied between these groups of studies, the more plausible explanation appears to be that inappetence and inanition were not behaving in the same way as they had apparently been in the earlier studies completed in Western Australia. There are a variety of possible explanations for this. The type of sheep being exported may have changed over time, and selection and management of export sheep was considered likely to have improved significantly over the intervening years, due in direct measure to the knowledge and recommendations arising from the work of groups in Victoria and Western Australia.

Mortalities recorded at arrival at the assembly feedlot were found to be significantly associated with body weight of sheep, distance travelled and with presence or absence of rejections at the time of arrival (Makin et al 2008). These findings were consistent with other reports from earlier studies.

During the B.LIV.0123 study, project team members were present at three assembly depots to post mortem every sheep that died and apply a standardised protocol to establish causes of death. There were very few deaths in the assembly feedlot periods that were intensively studied in comparison to the number of sheep that were processed (0.047% or 0.47 deaths per 1000 sheep). Causes of death included trauma (predominantly linked to the truck journey and appearing as deaths in the first 1 to 2 days after arrival at the feedlot), enteritis due mainly to Salmonella and a range of sporadic diseases and conditions including congenital disorders and urinary tract disorders. Enteritis cases tended to occur towards the end of the feedlot period (in contrast to trauma related deaths that were clustered at the beginning of the feedlot period). Risk of Salmonella outbreaks in assembly depots were potentially explained by factors associated with the level of shedding, environmental contamination and exposure of susceptible sheep. Over time as assembly depots were used for successive shipments, there was an increasing likelihood of progressively heavier levels of environmental contamination leading to a progressively increasing risk of a Salmonella outbreak.

Results from post mortems conducted during voyages as part of the B.LIV.0123 project and reported by Makin et al (2008) indicated that enteritis and inanition continue to represent the major causes of mortality in sheep exported live by sea, accounting for over 76% of diagnosed mortality that occurred during the voyage component of export. Enteritis was found to be the most common cause of mortality on board ships (34.4%), followed by inanition (23.9%) and enteritis/inanition (18.2%). In many of the cases that were displaying signs of both enteritis and inanition there also signs that animals had adapted to the export ration (pellets in the intestinal tract) indicating that persistent inappetence and inanition were less likely to be the primary causal factors leading to salmonellosis.

The two major causes of death on-board ship that were reported from B.LIV.0123 (salmonellosis and inanition) were the same as had been reported in earlier studies in Western Australia but the relative importance of inanition relative to salmonellosis was reversed in B.LIV.0123 compared with reports from Richards et al (1989) and Norris et al (1989b). It is not clear why there has been an apparent change in the relative importance of inanition and salmonellosis as causes of death with salmonellosis appearing to become more important and inanition less important. The two groups used very similar criteria to diagnose salmonellosis and inanition but differed in their approaches to the classification of animals that displayed signs of both enteritis and inappetence/inanition. These differences are considered likely to have contributed to the apparent differences in results but unlikely to be the major or only explanation for the change in relative importance over time of the two major causes of death. The two decades from the late 1980s has also seen a great deal of change in the live export industry and major improvements in standards across all segments of the industry. The overall mortality rate has reduced over time and a great deal of attention is paid to animal health and welfare throughout the export process. There have been changes in the type of sheep being sourced for export (fewer heavy, older wethers), changes in nutrition and management during assembly feedlotting, shorter assembly periods and earlier interventions to address perceived problems. In addition some changes may have increased Salmonella risk such as increases in throughput in assembly feedlots, more intensive sheep management and sheep movements and other stressors. All of these issues are believed to have contributed to the apparent differences in mortality risk and in risk factors between the two broad time periods of investigation. It is noteworthy that the results of all studies are consistent in identifying inanition and salmonellosis as the two major contributory causes to mortality through the assembly and voyage components of live export.

5.3 Causal factors

The causal relationships between salmonellosis and inappetence are complex and multi-directional.

Persistent inappetence may predispose sheep to disease such as Salmonella (Richards et al 1989; Norris et al 1990; Richards et al 1991; Higgs et al 1993), through a combination of changes to the rumen and other parts of the gastrointestinal tract that may allow increased survival and proliferation of Salmonella and through general debilitation that may increase susceptibility to widespread infection and septicaemia. This causal relationship sees inappetence occur prior to salmonellosis and predisposes an animal to salmonellosis. Animals with inappetence may then proceed to get salmonellosis or they may continue through to inanition and death from starvation without the added complication of salmonellosis. It is noted that inanition may also predispose an animal to a variety of other complications including metabolic changes and other infectious conditions that may also contribute to death. Higgs et al (1993) suggested that inappetence precedes and predisposes animals to infection with salmonellosis. This hypothesis is supported by Black (1996) based on observations on sheep exported from New Zealand. Black (1996) suggests that if inappetence and inanition are the major predisposing causes of salmonellosis in export sheep then preventive strategies should logically be directed at preventing inappetence and that this would then prevent salmonellosis.

However, salmonellosis can also predispose to inappetence and negative energy balance by two mechanisms. Firstly, salmonellosis causes inappetence with one of the first signs of clinical salmonellosis being depressed appetite. Secondly, negative energy balance can be exacerbated by salmonellosis as infection increases metabolic rate and demand for energy. Under this scenario, salmonellosis may occur without prior inappetence and animals with salmonellosis may proceed to die from acute disease or they may go on to show signs of inappetence.

Finally, either condition (salmonellosis or inappetence and consequent inanition) may occur in the absence of the other.

This complex and multi-directional relationship means that it can be difficult at times to determine the causal or chronological relationship between these two conditions (salmonellosis vs inappetence) in sheep mortalities during live export and as a result there may be difficulties in determining the relative importance of the two conditions.

More (2002a) divided salmonellosis in export sheep into two different syndromes. The first was described as ***classical salmonellosis*** and was attributed to overwhelming challenge of stressed sheep with virulent organisms resulting in acute salmonellosis (Figure 2). The second was termed the ***persistent inappetence – salmonellosis – inanition*** (PSI) complex and is depicted in Figure 3 (More 2002a). More (2002a) also suggested that while the clinical disease associated with salmonellosis under the two syndromes was identical, the causal webs for the two conditions may be slightly different and that these differences might have implications on control or preventive strategies.

Figure 2: Causal factors involved in classical salmonellosis (More 2002a)

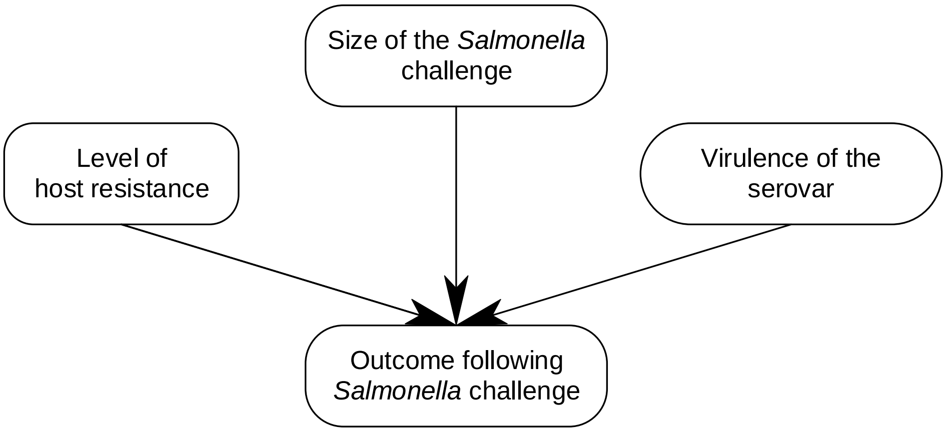
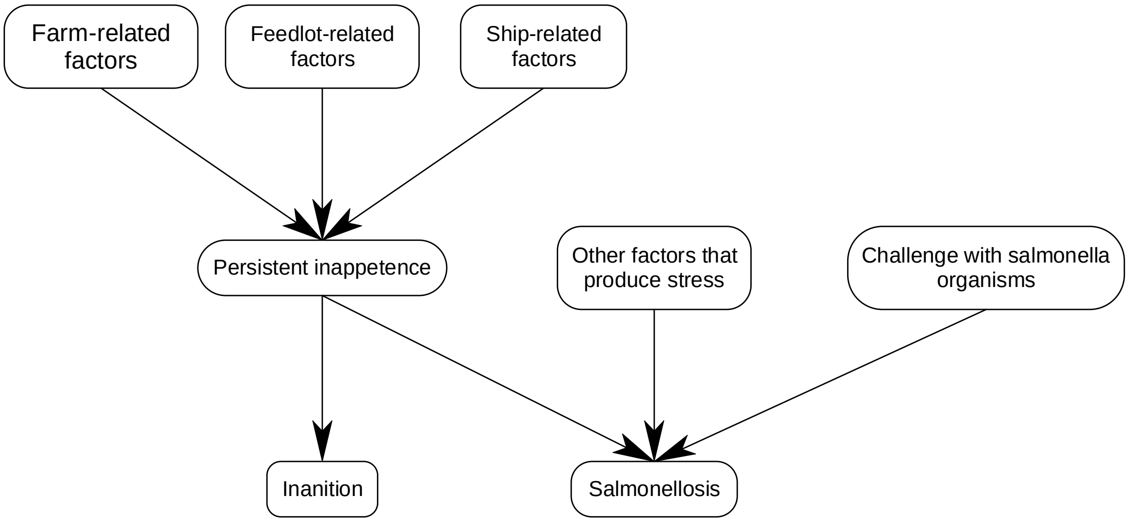


Figure 3: Causal factors involved in the PSI complex (More 2002a)

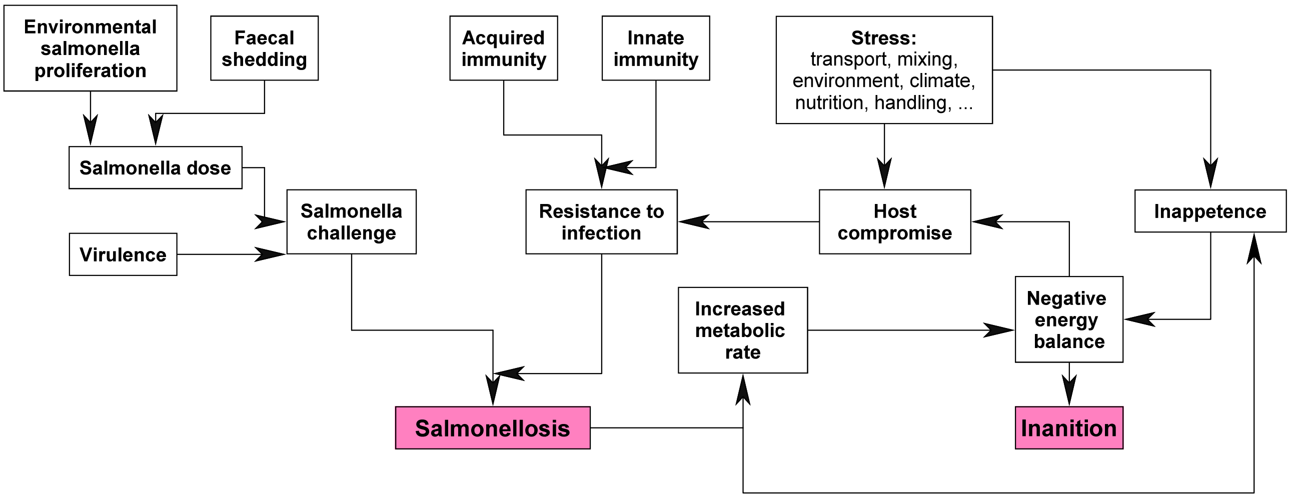


The major driver for the PSI complex was identified as persistent inappetence (More 2002a). Animals that did not eat as a result of the various stressors and changes that occurred during the period following departure from the property of origin were then considered to be at elevated risk of either dying from inanition or developing salmonellosis. In this causal web salmonellosis is identified as a spillover disease, dependent on prior inappetence in combination with Salmonella challenge.

The authors of this report present a slightly different explanation that is best represented by a single, unified causal web of factors that influence the risk of both salmonellosis and/or inanition in export sheep (Figure 4). All of the factors discussed under inappetence and salmonellosis are contributory causal factors in this unified causal web. Additional contributory factors could be incorporated into the diagram to provide more detail on variations or modifications for existing factors. An example of this can be seen in the depiction of two component pathways for immune capacity, labelled acquired and innate immunity. Acquired immunity is used to refer to immune response mechanisms that are based on immunological memory that is generally reasonably specific and may be very specific. Acquired immune capacity requires some form of priming either by previous natural Salmonella exposure/infection, or through administration of a Salmonella vaccination. In many cases acquired immunity may be sufficiently specific that vaccination with one serovar of Salmonella for example may not provide protection against infection with another serovar. Innate immunity refers to the range of non-specific or generic immune response mechanisms that allow the body to react rapidly to any infectious or foreign antigen challenge.

The outcome for a particular sheep that incurs some or all of the contributory causal factors illustrated in Figure 4 will then depend on the particular combination of intrinsic and extrinsic risk factors that might be present at individual animal, pen, consignment, ship, truck or farm levels. This means that individual animals in the same pen may have different responses and that there may be clustering of responses (correlation) at various levels due to similar effects of level-specific risk factors eg all animals on the same truck then share any risk factors associated with that journey and all animals in the same heavily contaminated feedlot pen will share exposure factors.

Figure 4: Unified causal web describing factors that may influence risk of salmonellosis and/or inanition.



The findings of mortality studies from the 1980s and early 1990s included a large series of investigations performed in Western Australia and additional work in Victoria. More recently (2005-2008) B.LIV.0123 has been associated with studies of sheep exported from Victoria, South Australia and to a lesser extent, Western Australia.

There are important commonalities and distinctions between these two broad groups of studies with respect to salmonellosis and inanition. All studies have noted that inanition and salmonellosis were the two most important diagnosed causes of mortality in exported sheep with risk of mortality being higher during the voyage than during the assembly feedlot period (though part of this may be due to the fact that voyages may take 20-30 days while sheep are only held in assembly feedlots for 5 to 10 days).

Richards et al (1989) noted that inanition was the most important primary cause of death, accounting for 32% of all mortalities over six voyages and was followed by salmonellosis (28%), trauma (11%) and inconclusive (13%). Salmonellosis was generally the most important primary cause of death in assembly feedlots while inanition was the most important during the voyage component of export.

Makin et al (2008) reported very few deaths in assembly feedlots during their investigation for those voyages where detailed post mortems were conducted to ascertain causes of death.

Furthermore, enteritis that was attributed to salmonellosis was the most important primary cause of mortality during the voyage, followed by inanition.

Both groups reported variation between voyages and consignments of sheep with respect to causes of mortality and these variations are likely to reflect the complexity of the causal web and the fact that different risk factors will be operating (presence/absence and strength of effect) on different sheep as well as at different levels of clustering (farm of origin, truck, pen, consignment, ship etc).

The relative importance of inappetence in the unified causal web as a driver of salmonellosis is identified as an area where there do appear to be important differences between the two broad time periods of study. The earlier works as described in this report have hypothesised that inappetence is the primary driver of mortality risk and that inappetence may lead in turn to inanition or to salmonellosis. Strategies addressing inappetence were then identified as the most important issue since if inappetence was the primary driver of both inanition and salmonellosis, then addressing inappetence would result in effective prevention of both major end stage outcomes.

Findings from B.LIV.0123 have suggested that salmonellosis may occur without prior evidence of inappetence or inanition, and that this pathway to salmonellosis may have an important influence on mortality risk.

The authors of the current report prefer to interpret the causal web for salmonellosis and inanition through a single interconnected web with two main outcomes as depicted in Figure 4. The importance of inappetence as a major driver of negative energy balance that leads to inanition and to increased risk of salmonellosis is strongly supported. The authors also contend that risk factors other than inappetence may influence the risk of salmonellosis and that salmonellosis may occur in sheep without prior evidence of inappetence.

The key conclusions of this interpretive assessment of literature with respect to salmonellosis and inappetence are as follows:

- A single, unified causal web is supported as the best way to conceptualise the factors contributing to risk of the two major end stage outcomes which are inanition and salmonellosis;
- Different combinations of risk factors (presence/absence and strength) are likely to be operating at different levels (individual sheep and various levels of clustering in time and space) both between and within voyages;
- Inappetence remains as a major driver of the risk of inanition and of salmonellosis;
- Drivers other than inappetence may result in salmonellosis in sheep without any prior evidence of inappetence.

The importance of these conclusions concerning causal factors is that strategies need to be implemented to address contributory risk factors across the causal web and not just in the area of inappetence.

6 Property of origin

There is considerable interest in understanding risk factors affecting mortality in export sheep that may be operating at the level of property of origin.
Detailed studies were made of lines of sheep (group of sheep of the same type or class and from the same farm) in the 1980s involving application of coded ear tags to identify lines as they were inducted into the assembly feedlot (Norris et al 1989ab, 1990). In several experiments marker

bars were used in assembly feedlots to allow identification of feeders and non-feeders (Norris et al 1989ab, 1990; McDonald et al 1988, 1990). There were large differences between lines of sheep and the percentage of animals identified as feeders or non-feeders suggesting that inappetence and feeding behaviour may be influenced by factors operating at the line or farm level (McDonald et al 1990) and evidence that prior exposure to pellets as lambs or hoggets was associated with fewer non-feeders under export feedlot conditions. However, repeatability of farm-level feeding performance was relatively low indicating that there was variability both between and within farms with respect to the ease with which sheep appeared to adapt to pelleted diets under export conditions (McDonald et al 1988). Mortality risk appeared to be clustered at the line level with 54% of mortalities occurring in 25% of lines and individual lines having up to 14% mortality (Norris et al 1989a).

There was no difference in mortality risk between sheep sourced from the property they were bred on compared to sheep sourced from dealers (Norris et al 1989b). There was evidence to suggest that dealer lines were less likely to be inappetent. While some crude associations between farm-level factors and mortality risk appeared to be important, multivariable models did not identify any significant associations between transport or farm-level factors and mortality (Norris et al 1989b). Factors that were assessed included region of origin, previous experience of mixing with other sheep, experience with trucking and supplementary feeding, distance trucked from home to feedlot and time off feed at arrival at feedlot. The results were affected by a low overall mortality rate and the relatively small sample size per line enrolled in the study that was dictated by the logistic requirements in tracking sheep across the entire study and performing post mortems on all deaths. Some associations were observed in some voyages and not in others and the lack of consistency between voyages also contributed to an inability of overall analyses to identify relationships.
Norris et al (1989b) concluded that difficulties in identifying factors that explained the line-level variation in mortality risk may have been due to inadequate statistical power to identify factors with low impact or failure to identify or assess risk factors that may be operating. The authors also commented on the fact that although the marker bar technique provided an effective method for identifying and distinguishing feeders and non-feeders, it was logistically demanding and not suited to commercial feedlot situations because of the cost and labour requirements.

A subsequent study was performed in the 1990s involving identification of lines of sheep at arrival at assembly depots over three years (1994-1996; Higgs et al 1999). Line level mortality rates ranged from zero to 28%. Of the 479 lines of sheep from 405 properties, 40% had mortality rates less than 1% and half of all deaths were derived from 14% of the lines. Mortality risk appeared to be repeatable at the farm level in that farms with higher mortality risk in one year appeared to be likely to present with higher mortality risk in subsequent years. There was also evidence of spatial clustering with higher mortality risk in farms that were located in the south-west region of Western Australia, an area of higher rainfall and longer pasture growing season, compared with more extensive pastoral areas to the north and east of Perth. The spatial association appeared to be explained more by growing zone than by rainfall. The authors hypothesised that the repeatable nature of mortality risk at the farm level provided opportunities for selecting farms from different risk levels for further study and also as a potential method of controlling risk. The authors did not directly assess transport distance but crude inspection of a plot showing location of high and low mortality farms suggested that the patterns of geographic distribution were similar for the two groups.

Makin et al (2008) collected data from 27 voyages between 2005 and 2008. There were 10,428 lines of sheep in the final database. Of these 6,826 (65.5%) had no recorded sheep mortalities. Of the 3,602 lines that had recorded mortality, 1,925 (53.4%) had a mortality rate of 1% or less, and 72% of all mortalities were traced to 16% of lines. These findings are also consistent with earlier reports from Western Australia that noted a small proportion of lines accounting for most of the mortalities.

Makin et al (2008) reported that pastoral sheep had significantly higher risk of mortality during the voyage than non-pastoral sheep. Sheep that originated in Queensland and that were transported to properties in southern New South Wales or Victoria before subsequently being transported to assembly feedlots were also found to have significantly higher risk of mortality than sheep that were non-pastoral and that did not originate in Queensland.

The results from the different studies discussed above support the suggestion that farm-level factors have an important impact on risk of mortality in export sheep. A number of studies reported that a high proportion of mortalities were associated with a small proportion of lines or properties. When there is very low background mortality this is perhaps not surprising and does not necessarily confirm that mortality events may be due to factors that are operating at the farm level. Individual lines or consignments may incur mortality events because of some particular combination of risk factors that may include factors operating at levels other than the farm such as during the transport from farm to assembly depot, during assembly depot and on-board ship. Transport related issues may be considered to be confounded with farm-level factors since issues such as distance or time travelled will be closely correlated with farm identity. Some transport related factors such as distance and time travelled may in fact be considered to be farm-level factors due to their close association with farm. Sheep from the same line then tend to be housed together in one or more paddocks in the assembly feedlot and this means that risk factors operating at the assembly feedlot level may be confounded with factors that might be operating at the farm level. The description by Higgs et al (1999) that mortality risk at the farm-level tended to be repeated year after year, i.e. that some farms had repeated episodes of either elevated or low mortality risk in different years, tends to support the hypothesis that there may be factors operating at the farm level. These observations are still potentially impacted by the cross-classified nature of clustering in lines of sheep being prepared for export.

Studies to date have not been able to identify factors that are operating at the level of the farm and that may explain farm-level variability in risk. There are a number of possible explanations for this difficulty.

7 Explanations for difficulty in identifying farm-level factors

7.1 Low levels of mortality

Mortality rates in live export sheep are generally low and in recent years have been less than 1%. There is expected to be a background level of mortality that is due to a variety of causes other than conditions such as salmonellosis and inanition, and other important identified causes of export mortality such as trauma. These include a wide range of sporadic causes of death that may include congenital and unusual acquired conditions that individually are responsible for very few cases each. Background mortalities are associated with conditions that are likely to occur in sheep regardless of whether they are being prepared for live export or not and often these conditions are not important enough to warrant preventive strategies or interventions. In many cases there is little that can be done to eliminate occasional deaths due to sporadic causes. Background mortality is not likely to be due to any common causal factor or factors and therefore may be very difficult to prevent other than through general strategies aimed at good welfare and animal handling practices.

Preventive strategies are often primarily aimed at minimising the occurrence and impact of conditions that may cause larger numbers of deaths. Occurrences of elevated mortality in export sheep (mortality events) have been uncommon in the past several years. The major causes of mortality events (salmonellosis and inanition) are still operating but currently appear to be causing low levels of mortality and there have been very few spikes or outbreaks of elevated mortality. When an outcome of interest (mortality) is relatively rare and there are multiple possible causes that may contribute to explaining the variability in outcome, it is very difficult to design an effective study that can achieve reasonable statistical power for identifying causal factors because of the very large sample sizes that are required.

Appendix 1 provides details of sample size estimation for projects aiming to investigate farm-level effects on mortality in live export sheep. Findings are discussed in brief in this section of the report.

Assumptions were made for input parameters in sample size estimation including:

- Background mortality rates were estimated to be 7 deaths per 1000 sheep based on the overall voyage mortality rate observed in B.LIV.0123.
- It was assumed that there were four times more lines with low mortality (consistent with background mortality) compared with lines that express high mortality.
- Sample size estimations were based on identifying risk factors associated with relative risk (RR) of mortality of 1.5, 3, 5 and 10. A RR=1.5 can be interpreted as indicating a risk factor that is associated with a 50% increase in the risk of mortality compared with the reference level, and a RR=5 is associated with a 5-fold increase in the risk of mortality compared to the reference level. Generally speaking a RR of 1.5 is at the lower limits of biological plausibility and may be consistent with more subtle or complex associations between the risk factor under study and the outcome. In contrast, where the RR is 3 or greater it indicates a very strong biological association.
- Intra-class correlation coefficients (ICCs) were incorporated into sample size estimates to account for lack of independence in a data structure where units (sheep) are clustered into lines. Sample size estimations were performed using a range of ICC values including zero (indicating no clustering and used as a reference level for comparison), very low level of similarity or clustering (ICC=0.05), low (ICC=0.15) and moderate to high (ICC=0.25). The impact of clustering is to reduce the effective sample size meaning that the actual sample size must be increased to produce the same level of statistical power.
- All sample size estimations used alpha=0.05 and beta=0.2. Statistical power is equal to 1-beta (0.8). If power=0.8, then the estimated sample size can be interpreted as the sample size required to achieve an 80% probability of obtaining a statistically significant result if the underlying association is significant.

Detailed results are provided in Table 2 of Appendix 1.

Risk factors that are subtle or more complex may be associated with a lower RR value and generally require a higher sample size to achieve the same power. The largest sample size is required for those scenarios where the RR is lower (1.5).

The impact of clustering is interesting. Where there is no clustering, there is no change in the total sheep numbers required when the scenario changes from sampling 50 sheep per line to 100 sheep per line. However, as the effect of clustering gets more pronounced (ICC larger), the most efficient sampling scheme is one that samples fewer sheep per line and more lines.

The scenarios that are considered most relevant are those that involve RR values of 1.5 or 3, and ICC values of 0.15 or 0.25. These reflect associations that are relatively subtle and allow for low to moderate effects of clustering. More powerful associations would be detected as well with these sample sizes. The results indicate a requirement for sampling from 1,050 to 1,750 lines of higher mortality sheep and more than 4,000 lines of sheep experiencing background mortality.

To put these requirements into context, B.LIV.0123 collected data on 27 voyages over a period of just under three years and in that time the project accumulated data on a total of 10,428 lines of sheep. If it is assumed that 20% of these lines were higher mortality lines then this produces an estimate of 2,085 higher mortality lines. Therefore a study that aims to collect data from as many as 1,750 lines of sheep that experience higher mortality, will need to be conducted over a large number of voyages (around 23 to 25 voyages) and will therefore require collection of data from all eligible voyages over a period of about three years. If the study were to collect data from ~1,000 lines of sheep that experience higher mortality, it would need to be conducted over about two years.

The study would need to collect detailed information on farm-level risk factors and be able to trace animals effectively from farm of origin right through to the end of the voyage. Studies to date have either collected detailed information on smaller numbers of lines or sheep (Norris et al 1989ab; Higgs et al 1991, 1999) or have collected less detailed information on larger numbers of sheep (Makin et al 2008). The major challenges in this scale of approach are associated with traceability (being able to link information collected on specific sheep during a voyage to a particular farm of origin) and collection of detailed information on farm-level risk factors from all farms that contributed sheep to the voyages under study.

7.1.1 Clustering at different levels

Sheep in the export trade are clustered at varying levels and the levels of clustering appear to change as sheep progress through the stages. For example, sheep are generally clustered in mobs on any given property and the same clustering may continue through transport from property to assembly depot. In assembly depots sheep tend to be mixed with animals from other properties and placed into paddocks or sheds (new level of clustering in the assembly depot) for the duration of the assembly period. Sheep are then regrouped as they board the export vessel and are clustered into pens. Clustering presents a range of challenges in field trials due to the lack of independence of measurements made on individual animals and the need to account for this during analyses.

Depending on the level at which a particular outcome is measured (individual animal versus pen or group level), there are a variety of analytical strategies that can be used to adjust the results for correlations or lack of independence. An important impact of clustering is to require larger effective sample sizes to achieve the same statistical power when compared with trials where all measurements are independent. Where outcomes are measured at the cluster or pen level, this can have a major impact on the required sample size since the sample size may then be measured as the number of pens of animals as opposed to the number of animals. The fact that individual animals can be members of multiple different clusters is termed cross-classification and accounting for cross-classification can further complicate analyses and reduce the effective sample size.

It is important to note that the presence of confounding and cross-classification (sheep belonging to different clusters at different levels) mean that some caution needs to be exercised in attempting to assign mortality risk to farm-level factors. The data from studies to date are consistent with variation in mortality risk at the farm level through findings such as the fact that most mortalities occur in a small proportion of lines of sheep. However, studies to date have not identified any particular farm-level risk factors that can explain this variability and it is possible that contributing factors explaining the apparent association may actually be derived from other levels (transport or assembly feedlot) and the appearance of farm-level association might then be explained by confounding and cross-classification. For example, sheep in the same mob are generally trucked together and experience the same weather conditions, as well as management factors such as curfews and a range of other stressors. There are a large number of potential stressors that may occur at any stage along the export process including stages other than the property of origin, including for example stressors such as feed and water deprivation, and social/behaviours/physical stress associated with mustering, yarding, drafting, holding, curfew and transport. Because of mob clustering statistical analyses could potentially associate adverse outcomes (that occur secondarily to various stressors) with property of origin even if the causal stressors may have actually been experienced at other locations. Caution is urged to avoid over-interpretation of potential causal factors since animal susceptibility to adverse events and occurrence of adverse events may involve intrinsic factors (individual animal or mob variation in immune capacity or genetic predisposition to diseases for example) as well as a complex array of factors that may be operating at different levels.

7.1.2 Animal traceability

Animal traceability has been a major issue limiting the ability of field studies to investigate farm-level factors that may be influencing mortality risk. Studies from the 1980s and early 1990s described the application of individual ear tags to animals enrolled in studies to allow mortalities to be identified and traced back to property of origin (Norris et al 1989ab; Higgs et al 1993, 1999). Makin et al (2008) relied on industry records to try and trace sheep and described the difficulties in tracing animals to property of origin.

Currently sheep are generally identified at the mob level or at the level of property of origin and not at an individual animal level. Once animals arrive at an assembly depot they are often sorted by sex, breed, type and weight and combined with animals of similar class that may come from different properties. Sheep are sorted on arrival and often divided into multiple paddocks based on criteria relating to market specification. Sheep from multiple different lines may then be combined into one paddock depending on carrying capacity of paddocks. It is generally possible to link contributing properties of origin to particular assembly depot paddocks or sheds but the relative contributions of particular properties to any specific paddock or shed is difficult to determine and over time, as animals are moved or sorted again, it becomes increasingly difficult to determine the contribution of property of origin for any particular feedlot paddock. When sheep are transported from the assembly depot to the ship for loading, there is additional mixing of animals of similar classes and it is not possible to effectively trace animals from property of origin to specific pens aboard a ship.

It is important to note that there are two broad components to the issue of traceability. The first refers to the ability to be able to effectively identify an individual animal and link it to a consignment or line (accurate identification of individual animals is critical for estimation of a numerator or count of affected or dead animals for any specific line). The second refers to the ability to be able to maintain a count of the denominator or number of animals at risk at a particular point in time and for a particular level or unit of clustering. At the time that sheep arrive from property of origin to the assembly depot the count of animals unloaded and accepted for assembly feedlotting is an accurate estimate of the denominator of animals for any particular line. Animals may be lost (die or be culled) during the assembly depot process right up to the point when animals are loaded onto the export vessel. It is not common for feedlot operators to record details of losses in such a way that denominators can be adjusted and accurately maintained at the point of loading the export vessel. For very large lines of animals, failure to be able to adjust denominators for looses due to deaths or culls may not be critical since the change in dominator may be trivial. However, for smaller lines and for occasions where a high percentage of a particular line may be culled, the change can have a major impact on estimation of mortality risk. Investigation of risk factors requires accurate identification of numerator (count of affected or dead animals) and denominator (count of animals at risk) for each line of animals. This information is then coupled with information on risk factors to assess associations between risk factors and outcomes of interest (mortality rate for example).

If an animal dies and is then examined, the ear tag or brand may be able to be used to link that animal to a consignment or a property of origin. Makin et al (2008) reported that between 57% and 84% of mortalities on board export ship were able to be identified and traced back to a consignment or line in the assembly depot. The combination of variable traceability of individual animals on board ship and difficulties in maintaining an accurate record of animals at risk of mortality means that it is very difficult to accurately monitor mortality risk by property of origin using existing industry data.

There appear to be two broad approaches to this problem. The first is to use an approach that involves applying individual animal identification to animals that are to be followed during the study. This approach was used in earlier studies in Western Australia as exemplified by publications from Norris et al and Higgs et al. A major constraint with this approach is the additional cost involved in applying individual animal identification and the likelihood that labour constraints would mean that only a limited sample of animals would be identified and studied as was done in the earlier studies. Sample size estimations described earlier in this report indicate that all animals need to be followed and therefore this approach is unlikely to be effective.

The second approach is to work with industry to improve routine traceability by improving the way animal and mob identification information is collected and managed throughout the live export process. The goal would be to achieve the ability to accurately link any individual animal with its property of origin and to be able to accurately identify the total count of animals for each line at two or three key points along the chain including:

- Time of arrival at assembly feedlot (already being done)
- Time of loading of ship at end of feedlot and start of voyage (not currently being achieved)
- Time of unloading of ship at destination port (less essential but would be useful)

7.1.3 Complexity of the causal web

A large number of causal factors may be operating at different levels to influence risk of mortality in individual sheep and in clusters of sheep. These have been discussed briefly in earlier sections of this report. It is possible that different combinations of causal factors may be operating on sheep within mobs in the same ship and that the strength of particular factors may also vary within the same shipment as well as between shipments. Variability in presence/absence and strength of multiple causal factors both between and within voyages means that it is likely to be very difficult to identify particular causal factors. Confounding and cross-classification are also adding to the complexity of the causal web.

A complex causal web does not mean that studies cannot be done to identify causal factors or to develop a clearer understanding of the roles and interactions for various causal factors. However, it does mean that care will be needed to ensure that traceability, large sample size and valid representation of risk factors at different levels will be necessary to ensure that a causal web and the contribution of various risk factors can be determined.

8 Other relevant material

8.1 Previous workshops

Workshops have been held in 2008 and in 2006 in Perth to discuss aspects of inappetence and/or inanition and salmonellosis, and to identify possible questions or information gaps that may be used to guide future research projects.

These workshops have already influenced research activity principally through the development of tender documents calling for expressions of interest to focus on particular research questions or topics.

A summary of potential research questions that were identified in the 2008 workshop in Perth is presented in Table 1. A number of these questions are particularly well suited to investigation in controlled or experimental trials designed to investigate appetite and inappetence in sheep under conditions simulating assembly depot or voyage.

Other issues may be better suited to investigation in field trials that may include observational studies designed to follow relatively larger numbers of sheep through the natural export process and using predominantly existing systems and infrastructure.

There are also questions that may be best investigated using either experimental trials or field trials where interventions can be applied to some sheep. Examples of this include vaccination against Salmonella, evaluating feed and water additives to prevent or treat salmonellosis, and trialling a shortened or absent assembly period.

Others may be investigated using a combination of approaches. For example, observational studies may be used to identify properties with high and low risk of mortality and then these properties investigated in more detail using follow-up studies such as sourcing sheep from these properties for use in experimental trials to look for differences in appetite or disease responses to stress and/or Salmonella exposure.

Table 1: Summary of potential research questions arising from 2008 meeting in Perth

High priority	Medium priority
Factors controlling appetite & impact of on these of live export processes	Comparison of particular risk factors
Pellets - consistency, palatability	Effect of curfew
Identification of inappetence	Compare eastern vs western export systems
Management or treatment of inappetence - making sheep eat	Performance of traded sheep vs home bred
Incorporate different groups of sheep in trials assessing inappetence	Transport related issues
Field trials to investigate apparent farm-level differences	Weather during voyage
Better use of existing data, personnel & record keeping/collection	Trough design
Use of NLIS capability	Vaccination against salmonella
Assess potential risk factors: curfew, sheep type/age	
Using a risk-based approach to R&D	**Low priority**
Consider trialling a shortened feedlot or no feedlot period	Hormonal manipulation of appetite control
Vaccination against salmonella	Why sheep resume eating on board ship
	Field trial issues
	Fatness in second half of year
	Industry monitoring - using existing data & systems

The 2006 workshop produced broadly similar areas that were considered to warrant further investigation. There was interest in using data from routine voyages and from B.LIV.0123 (Makin et al 2008) (that was then in its first year of operation) to identify groups of sheep with high and low risk of mortality and then use that information to direct further more detailed investigation, both of source-farms and by enrolling sheep from those groups in experimental trials. Examples of these sorts of activities included:

- Collection of information from source-farms either by questionnaire or by field visits and sample collections. These activities could be done retrospectively to link contemporaneous farm-level information with mortality information from already completed voyages, or could be designed as separate studies to investigate particular hypotheses developed from analyses of completed voyages.
- If farms could be categorised as low or high risk for mortality based on analyses applied to completed voyages then it might also be possible to source additional sheep from selected farms for further experimental studies to look in more detail at animal or even molecular level factors that may be associated with farm-level mortality risk under export conditions.

Farm-level factors that were tentatively identified as being worthy of further assessment as potential modifiers of appetite included age, season, fatness, temperament, grazing history, management, handling frequency and transport factors.

8.2 Prior reports on risks and best practice

Previous reports have made a number of recommendations concerning risks of salmonellosis and best-practice in live sheep export feedlots (More 2002ab; 2003; Makin et al 2008; Barnes et al 2008). A number of these recommendations remain relevant for industry to both reduce risks of inappetence and salmonellosis and also to develop and implement systems that allow routine collection of data that can be used to identify problems both for ongoing management purposes and as a component of integrated research programs. The use of a risk management framework is supported to develop practices that are likely to reduce risk of the major causes of mortality in export sheep (inanition and salmonellosis).

A risk management framework should incorporate the following characteristics or attributes:

- Reflect current knowledge concerning risk factors that may be associated with mortality in export sheep and incorporate some form of weighting to account for variability in validity of such knowledge (higher weighting applied to science-based information and lower weighting to anecdotal reports for example).
- Measures must be practical and cost-effective and be able to be implemented into routine management of the live export trade. This requires detailed understanding of management practices in the industry.
- Measures should be capable of being monitored and reviewed against implementation and performance objectives or standards. This ensures that industry can identify effective and ineffective measures and modify practices accordingly.

9 Proposed activities

The strategy outlined in this report involves the development of an integrated approach that is based on broad, industry-wide monitoring and surveillance of mortality coupled with additional studies – particularly experimental or intervention studies – to assess the impact of management and preventive or treatment options designed to further improve health and welfare. The broad approach would deliver ongoing monitoring and quality assurance capability as well as flexibility and responsiveness. It would allow early detection of problems, have effective feedback and capacity to provide monitoring and assessment of any interventions, and would allow investigative capacity to be directed at specific problems or areas of interest to industry stakeholders. It would build on the substantive and valuable background of research and knowledge about the industry that has been accumulated to date. Broad industry-wide monitoring would generate hypotheses and inform the design and conduct of specific experimental studies. Results of both types of studies would in turn inform management practices.

The objectives for the current study describe the development of a scientifically sound methodology and robust implementation plan to address the current gaps in knowledge regarding the reasons for variation in the number of mortalities between farm groups. The objectives also described the development of a budget.

It is logical to expect that all proposed activities be robust. The term robust is interpreted as indicating that strategies and methods are based on common sense and are resistant to error or variations in application or operation. These characteristics help ensure that strategies are likely to be adopted by various industry operators, and are able to collect high quality data and information in a standardised manner with high compliance (little missing data) and consistency (similar data and methods across different companies and individuals). Robustness is particularly important when activities or operations involve multiple people from different companies or organisations as illustrated by the concept of an integrated information management system that may be implemented across the export industry to improve routine data collection from all voyages. In some cases, where the proposed project aims to deliver something that will then be implemented in an ongoing way by industry, the term robust can be used to describe the development activities as well as the way the deliverable (an information management system for example) will be expected to function in the future.

Scientifically sound methodology is also important and refers to multiple dimensions of the proposed activities. The first is that the background and justification for the proposed project aim and objectives must be based on sound science including for example review of current knowledge through published scientific literature, identification of knowledge gaps that are important for the particular outcomes of interest. A second dimension is that the particular methods being proposed to achieve the objectives must be based on sound science. Finally, where a project is intended to deliver something that will then be implemented in an ongoing way by industry (such as an information management system), the system should be capable of itself providing scientifically-sound data and information for industry benefit.

The current report has not developed budgets for proposed activities. The report has focused on developing material that can be used as the basis for developing a Terms of Reference document should the ideas be progressed into projects. It is assumed that those projects for which a Terms of Reference document is produced, would then be advertised in an open call for expressions of interest. A detailed budget would then be expected to accompany any expression of interest. It was felt that attempting to estimate a budget for the projects as they are presented in this report would not serve any useful purpose and may mislead potential applicants as they prepare a submission in response to a call for expressions of interest.

9.1 Development of an integrated information and management system with monitoring and surveillance capacity

9.1.1 Background and introduction

The live export industry is heavily regulated and has a number of monitoring and reporting obligations concerning animal health and welfare. The authors believe there is potential to adapt routine industry practices to facilitate industry management of regular shipments (make things work more smoothly and effectively for industry operators), and collect better information for industry purposes including contributing to effective quality assurance and research as well as meeting regulatory reporting requirements to AQIS.

An example of this approach has been seen in the development of the prototype information management system as part of B.LIV.0123 activities (Makin et al 2008). A similar approach will be applied in a recently commenced project in the cattle export industry (W.LIV.0252) to incorporate ongoing data collection concerning causes of mortality into routine veterinary activities on export ships so data can be collected in a sustainable ongoing manner. Incorporation of data collection systems into routine activities is identified as the most cost-effective and sustainable way to ensure good coverage across the entire industry and to allow monitoring to continue in an ongoing way. Achieving this will require strong industry support for the approach and requires that the systems be purposefully designed to meet the requirements for day-to-day management of export operations as well as delivering data and information for broader strategic and research objectives.

It is also useful to contrast the approaches employed in B.LIV.0123 and those planned for W.LIV.0252. The sheep project (B.LIV.0123) involved mobilisation of a project team to attend assembly feedlot periods and conduct sampling and collect data. In addition project team members accompanied voyages and conducted post mortems. When the project was completed these activities stopped. The project has produced a prototype information management system (IMS) that is still being used by some industry operators but this system has no ongoing mechanism of support for maintenance and further development so is not expected to be maintained over time. Involvement of project team members in data collection and investigation activities ensured that procedures were done according to protocols and that data were collected. However, this approach can be more expensive and is based on an assumption that outcomes of interest will occur during the project time frame. In fact there were very few mortality events in the sheep trade during the conduct of B.LIV.0123 and this meant that a great deal of effort was expended to collect data during a period when mortality rates were very low and were predominantly caused by background and sporadic causes of death.

In contrast, the proposed approach being implemented in W.LIV.0252 is to use project team members to develop specifications, protocols and systems but then to have most of the collection of data, samples and information performed by AQIS Accredited Veterinarians (AAVets) performing their routine duties during export voyages. A PhD student will examine the samples and analyse data for the project. Sustainability is a key issue. This project is being carefully developed to ensure that the systems continue to be implemented by AAVets beyond the lifespan of the project. In addition it is expected that additional standardisation of systems will have been developed for use in pathology laboratories and that ongoing work will be conducted on samples to confirm the causes of death. W.LIV.0252 is considered to be much more robust and sustainable than B.LIV.0123. It is also likely to have a more narrow focus in part because the project has to be designed to be very simple and to be carefully standardised because the people doing the data collection are industry AAVets and not project team members.

In an environment where there is a high likelihood of mortality events and other adverse outcomes of interest occurring over time, a more intensive, costly project with highly trained project personnel directly involved in data collection and sampling, becomes more effective because it can allow more detailed data collection and can be focused for a short period of time and collect high quality data that can be analysed and the findings applied more generally. In an environment where the outcomes of interest are uncommon (or rare) and highly variable and perhaps unpredictable in occurrence, a high-cost, short time-frame project with intensive investigation and sampling becomes a much more risky proposition because of the risk of having the project perform detailed investigations when there is little happening of interest. Under these situations, the preferred approach is to design a low-cost, longer-term, sustainable capability that can be used to monitor the outcomes of interest over time, identify problems early and provide high quality but simple data to describe patterns and generate hypotheses. When there are no problem events, this approach provides excellent general monitoring for quality assurance purposes. When problems occur it provides valuable descriptive data and information, early detection and can inform more detailed studies.

The combination of a sustainable, broad monitoring capacity (scanning surveillance in a biosecurity sense) and targeted, more detailed studies to investigate particular problems or test specific hypotheses is considered to offer the best mix of capacity when adverse outcomes are uncommon and unpredictable. This is the mix of attributes that is considered to be robust and scientifically sound in investigating property of origin effects associated with mortality in export sheep.

Characteristics or attributes of an integrated system include:

- Single data entry where-ever possible to avoid duplication of data entry and associated risk of errors.
- Linkage with the NLIS database to facilitate traceability of animals and particularly linkage of lines of animals in the export assembly feedlot or on ship with PIC information to allow identification of the property where the sheep were transported from when they travelled to the assembly feedlot.
- Linkage with other information would be very useful including the ability to spatially locate vendor properties so that geographic information system (GIS) capability can be incorporated into monitoring efforts.
- Standardised approach to data recording to minimise errors and variability in data.
- Integration across different sectors of the export industry (buyers, assembly feedlot, ship) so information entered from one sector can be accessed by other sectors.
- Capable of providing the routine day-to-day data and information that exporters and other industry operators require for management of their business operations. This includes an ability to be tailored to suit requirements of sectors and operators to fit in with particular management styles or objectives, and an ability to securely control access at multiple levels for each component of the system.

- Capable of being used to generate reports for various stakeholders including AQIS reports for example.

Traceability of animals is critical in this process, including the ability to link individual animals to a property of origin and particularly the property they were located on and transported from, when the animals were transported to the assembly feedlot. Traceability also refers to the ability to reconcile denominators (total number of sheep in a line). The traceability component of the system is dependent on being able to use an observation from an individual animal (typically an ear tag) in order to trace that animal to a mob or line at receival into the assembly feedlot and therefore to a vendor property. This characteristic would appear to be consistent with the current National Traceability Performance Standards.

It is understood that from 1 January 2009, all sheep should have NLIS compliant ear tags prior to movement. These tags should allow tracing of animals to a receival line and to a vendor. When an animal dies, retrieval of a NLIS ear tag should then allow an accurate count of mortalities for each line (numerator for a mortality summary). Experiences from B.LIV.0123 indicate that there have been problems in the past over matching ear tags retrieved from bodies to particular lines of sheep. The reasons for such problems have included recording or transcription errors, multiple ear tags and retrieval of a non-informative tag while other tags may have been left on the body, and insufficient information able to be retrieved from the tag to match to any one line. Mortality counts (numerator) are best expressed as some form of rate or risk and this requires a denominator (count of animals at risk of dying) for each line. The most accurate count of a denominator at the line or farm-level in the live export trade appears to be based on counts of animals received at the assembly feedlot. Deaths during the feedlot period can then be identified through tag retrieval and the denominator for a line adjusted to be accurate at the time of loading of the ship. However, there are occasionally reasonable numbers of animals that are culled from an assembly feedlot due to inappetance or disease conditions and these animals may not be recorded so that denominators can be adjusted. In Western Australia where it is common for sheep that are removed from one shipment to be held over in the feedlot and placed on a subsequent shipment, it can get quite confusing since these animals may not be accurately accounted for at the line level in either shipment. Traceability requirements for an effective monitoring system for the export industry would require the following capabilities:

- Ability to link an individual animal to a specific line of sheep at any time during the export process, preferably by tag retrieval.
- Reconciliation of denominators or total counts of animals in each line at two key points with consideration being given to reconciliation at a third point:

- On arrival at the assembly feedlot
    - Involves recording identification information (NLIS identification, PIC etc) and counts for all sheep entering the feedlot as is currently done.
- On loading of the ship, including:
    - Recording the number of sheep and NLIS identification (line identity) for all sheep that are removed from a shipment after receival and up to the time of loading the ship.
    - Recording the number of sheep and NLIS identification for all sheep that are added to any shipment and that are not recorded in the receival records. These would be sheep that have been held over from a previous assembly period and that are already in the assembly depot but are then not counted in the receival records for the next assembly period.
- On unloading of the ship at destination ports

A prototype information management system was developed during B.LIV.0123 and implemented in assembly feedlots and on some voyages and has been well received by industry operators (Makin et al 2008). Some operators have continued to use the system beyond the lifespan of the project which reflects the effort undertaken to design a system that made day-to-day management easier for operators as well as provided data for a research project. The system developed for B.LIV.0123 was relatively crude, has no ongoing means for support and further development, and is not suitable for development into a product that can be implemented across the industry. It has provided valuable insight into the requirements for a system to be effective and useful for operators as well as how other data and information needs or value can be incorporated into a system to meet other industry needs (quality assurance, strategic planning and research). In the time since B.LIV.0123 began in 2005, the NLIS program for sheep has been fully implemented and there has been a growing awareness of the capacity to leverage additional value from the NLIS database for industry benefit.

B.LIV.0123 incorporated a PhD student project and funding for additional researchers who attended assembly feedlots and accompanied voyages and performed post mortems and collected, entered and managed data. The prototype information management system developed as part of B.LIV.0123 provided the ability to collect data on all shipments and not just those that the research team attended. However, there were key pieces of information and data that were lacking from those voyages that were unaccompanied by research team members. These particularly related to the application of standardised approaches to post mortem of all dead animals during the assembly feedlot and during voyages. Planning for W.LIV.0252 incorporates development of standardised approaches to collection of data and information on morbidity and mortality as part of routine shipboard veterinary activities so that this information can be collected on a routine basis during industry operations and is not dependent on specific research project funding or on the presence of one or more funded researchers. This approach is seen as the most effective way to ensure broad coverage and collection of data relating to health and welfare performance into the future. This is particularly important given that mortality rates are very low and that spikes of elevated mortality are uncommon or rare and are unpredictable. A system that covers all shipments and all animals and that is incorporated into routine day-to-day management provides the ability to monitor events continually and to identify and respond to problems when they arise and contributes to ongoing research in a very sustainable manner.

There is an opportunity to realise these benefits for individual operators and for the industry through the development of an integrated information management system that meets all of the performance criteria discussed above.

The recommended approach to development of an integrated system for monitoring and surveillance of mortality (and to a lesser extent morbidity) involves building on the findings from B.LIV.0123 and the experiences from W.LIV.0252.
The characteristics described above are all considered important with respect to the qualities of robustness and science-based methodologies. The approach outlined in this integrated information management system is based on sound science and particularly on the importance of having accurate measures of numerators and denominators for outcomes of interest such as mortality rate. Current practices for recording animal identification records do not allow accurate recording of these two key measures. Applying animal identification tags to a subset of animals under a specific experimental protocol is unlikely to deliver sufficient data to achieve reasonable statistical power. Development of a robust, industry-wide approach to ongoing collection of data is therefore seen as a scientifically sound approach to ensuring ongoing recording of complete and high-quality data.

It is important to note that attributes of robustness and sound science for this project apply most particularly to the way the intended deliverable (information management system) may function and the nature of the data and information it should be able to deliver, and less to the particular methods and steps required to achieve the objectives for this particular project. This is because the project outcome is not to resolve specific hypotheses concerning farm-level risk factors and health outcomes in export sheep, but is intended to provide important, ongoing functionality for industry to allow future routine collection of data from all voyages to underpin and support a range of subsequent industry activities including future research as well as quality assurance and industry planning.

There is considerable interest in developing more efficient and effective ways of managing and using information relevant to the export industry and this is evidenced by the interest in the prototype management system produced during B.LIV.0123 and similar activities underway in W.LIV.0252. There is potential to broaden the scope of an Information Management System to cover all species of animals that are exported with a particular focus on sheep and cattle for example. Many industry operators are involved in exporting multiple animal species and there is logic and economic rationale in combining efforts to produce a single product that can cover multiple species. It is acknowledged that there are quite different data structures between sheep and cattle, most notably related to the individual animal identification requirements associated with NLIS for cattle and the mob-identification processes currently being applied for sheep and goats. However, it is considered possible to develop an umbrella system with a common user-interface and appearance that may have underlying modules or components capable of handling any species.

9.1.2 Aim

To develop an integrated information management system capable of being used by industry operators for day-to-day management of export operations and of delivering data and information to industry for more strategic purposes including quality assurance, research and industry planning.

9.1.3 Objectives

There are a number of objectives that contribute to the broader aim including:

- Identification of options for linking industry systems with the NLIS database and with other databases that may be relevant to the export industry (such as spatial land parcel information in various jurisdictions).
- Developing appropriate manuals and procedures for use in the live sheep export industry that outline standardised approaches to collection of health and welfare data including investigation of sick and dead animals and a standardised approach to post mortem of dead animals. This involves building on previous research in the export industry and particularly on B.LIV.0123 and W.LIV.0252.
  - A veterinary export handbook for sheep that contains descriptions for common diseases that occur in live export sheep including diagnosis, management, treatment and prevention. The handbook will also describe procedures for investigation and data collection from sick and dead animals including how to perform a post-mortem examination of animals that die during a voyage. An important part of the handbook will be development of standardised terminology for use in describing animal health and health disorders including morbidity and mortality.
  - Define data requirements necessary to be able to describe mortalities and morbidities that occur during the live export process and particularly during the voyage. This will include data to describe the vessel, voyage (load date, climatic and sailing conditions, destination, load plan, etc), denominator or line of sheep, 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 post-

mortem examination). It is expected that capacity to deliver reports as defined in the export standards or required by AQIS will also be incorporated into the system.

- Define data requirements for collection of animal and line identification data to facilitate tracing individual animals to a particular line and a line of animals to the property of origin (property the animals were housed on prior to being transported to the assembly feedlot). These data are expected to be based where possible on NLIS data but alternative systems may be considered.
- Systems should incorporate the capacity to collect samples from animals that die during a voyage and import those samples back into Australia for examination by veterinary pathologists to establish the cause of death. It is expected that this may be implemented only in cases where specific projects are designed to investigate causes of death or where there is particular interest in an unusual event or occurrence of mortality.
- Review, clarify and define 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 other objectives.
- Complete a training needs assessment and describe options for delivery of suitable training material and training activities for accredited veterinarians and stockpersons to support the ongoing functionality of an industry information management system.

• Completion of a document incorporating specifications for an industry supported information management system to meet industry needs for day-to-day operations and more strategic needs including quality assurance, strategic planning and research and development. This process would incorporate options for development and ongoing support and maintenance of a commercial web-based software system. It is expected that this will build on experiences of the B.LIV.0123 project and the prototype IMS produced during that project.

- Description of data and information flows under current management systems used for routine day-to-day management and for compliance and regulatory purposes.
- Completion of a specification document for an information management system that is capable of meeting routine needs and of providing valid and reliable data on mortalities and animals at risk (denominators) at the line level for all sheep on board a particular voyage.
- Description of options and associated budgets for development including software and hardware platforms and ongoing support and maintenance. It is expected that emphasis will be placed on a web-based system and that hand-held data capture devices would be considered for data entry on-board ship and at assembly feedlots.

• Development of an industry supported information management system (IMS) capable of supporting ongoing monitoring and surveillance needs as well as industry quality assurance and routine and strategic management.

- Development of a prototype system that could be applied on a number of pilot voyages.
    - Finalisation of prototype IMS for installation
    - Development of help files, operating manual and training package for users
    - Analyses of collected data on morbidity and mortality including assessment of traceability, data quality and descriptions of morbidity and mortality by line.
- Final development of an industry information management system
    - Management and incorporation of feedback from users and industry stakeholders from pilot testing of a prototype

- Completion of a commercial-type application product for implementation across industry
- Implementation of systems for ongoing review, development, maintenance and support
- Implementation of systems for ongoing analysis and reporting of data collected over time

9.1.4 Methods

The project will involve consultation with the NLIS committee within MLA and with state jurisdictions responsible for NLIS activities as well as with industry through workshops and one-on-one consultation with industry operators.

Objective 1 will involve completion of a report that describes desired functionality for a system to be able to access data from relevant databases, constraints that limit access and functionality (including relevant legislation) and options for addressing those constraints. The likely constraints to accessing NLIS and spatial databases for industry purposes other than those mandated under legislation designed to facilitate emergency response to a disease outbreak, mean that it is logical to complete this component activity separately and prior to considering other objectives associated with this monitoring system. If it is not possible to link an industry system to the NLIS database or other relevant databases then it does not negate the value of an industry monitoring system but alternative approaches will be necessary to collect relevant data. These outcomes will have flow-on effects on subsequent Objectives.

Objective 2 will involve completion of a report delivering against each of the component objectives approaches. In addition it is expected that the veterinary export handbook will be produced in electronic form and in bound form suitable for use within day-to-day operations. It is expected that the project team will review the prototype system produced and implemented within B.LIV.0123 and the activities that have commenced under W.LIV.0252.

Objective 3 would be informed by the reports from Objectives 1 and 2 as well as those from other projects including B.LIV.0123 and W.LIV.0252. Activities require the involvement of a team incorporating skills and experience relating to industry operations and epidemiological studies of health and welfare outcomes as well as computer programming skills. The outcome is expected to be a report containing detailed specifications describing the functionality and structure and relationships for a web-based system capable of being implemented at multiple levels across the industry. Estimates of costs for development of a prototype as well as for completion of a commercial product and ongoing support and maintenance/development costs would be incorporated in the report. It is likely that further consultation will be required during the course of these activities to ensure the specifications are compatible for existing systems and with end user requirements and capabilities.

Objective 4 is dependent on feasible and practical options having been identified in Objectives 1 and 2 and on industry support for the proposed specifications completed as part of Objective 3. Development of a system would involve one or more computer programmers and a project manager and would be heavily based on the specification documents arising from Objective 3.

Application of the system in a small number of pilot voyages will require a project team capable of implementing the system as well as provision of additional outcomes related to training and instruction in system use, development of user manuals and help files and provision of support. It is also important to note that support will be required for training in animal-based activities (investigation of sick animals and post mortem of dead animals) and for collection and analysis of data entered into the system. It is expected that some analytical and reporting routines will be programmed into the system in an automated or semi-automated manner but that other analyses are likely to be of value to industry as well.

The second component of Objective 4 involves incorporation of feedback from pilot voyages and industry experiences into modifications of the system and then roll-out of a system for industry use. These activities are expected to also involve training as well as ongoing support and maintenance costs.

9.2 Experimental studies to investigate inappetence and inanition

9.2.1 Background and introduction

It is understood that a recent application in this area has been approved for research funding[c]. The application describes a relatively large-scale investment in the capability to electronically track larger numbers of animals under experimental conditions using radio tracking and purpose designed tags – placed in the ear or as wearable collars. The methods offer potential to record relatively simple information about feed trough access to provide information on which animals feed and which ones do not and allow investigation of factors associated with inappetence.

Alternative technology - understood to not be available currently – has the potential to record detailed spatial and positional data on individual animals within a mapped area, such as a feedlot shed or pen. The more detailed capacity is capable of providing information about the activity of animals, such as whether an animal went to a feed trough or water point, the time spent at each area, as well as three-dimensional data that can be interpreted as standing, lying, and moving around. Such a system could then be used to indicate animals that were feeding and drinking, which troughs they used, what other activities they performed, which other animals they associate with, and therefore provide a great deal of behavioural data on individual animals. The same system could also be used to evaluate interventions or treatments.
The establishment of this methodology is certain to represent a long term investment with many years of subsequent experiments being able to take advantage of the initial investment in technical capacity. The methods have the potential to facilitate a great deal of research into appetite control and inappetence as well as other areas of interest for the export industry associated with animal behaviour, welfare and health. The enhanced recording capacity and level of detail should allow hypotheses from earlier studies of factors that may be associated with inappetence to be revisited and for additional studies to further explore drivers of appetite and potential methods for reducing the risk and impact of both temporary food removal through curfew and transport and of inappetence. Investigation of factors such as feeding preferences, pellet composition, pellet dust, trough design and access to water have all been identified as potentially useful project ideas that could be studied using the experimental capacity that will be established as a result of this investment.

This report does not propose any further actions in relation to these activities given that the research application has been approved and is being progressed separately to this report.

9.2.2 Aim

To develop capacity to undertake experimental studies under conditions simulating assembly feedlot or voyage conditions and that allow detailed investigation of animal physiology and behaviour for the purposes of understanding factors that influence appetite and morbidity risk in export sheep.

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[c] Backgrounding and feedlotting strategies to address Inanition in the Livestock Export Industry. Application by Anne Barnes et al (2008).

9.3 Investigation of farm-level factors associated with mortality risk

The use of field observational studies to investigate farm-level risk factors that may be associated with risk of mortality has been discussed earlier in this report. These approaches are likely to be successful only if:

- they involve reliable tracing of animals to line and to farm of origin (property that the sheep were on immediately before they were transported to the assembly feedlot)
- they involve collection of data from large numbers of lines of sheep over a long time period
- farm-level risk factors are truly responsible for variation in mortality that appears to exist at the line-level
- data/information is collected on the right risk factors to facilitate detection

Because of clustering of sheep in lines there is considered to be benefit in a multi-stage approach to sampling with a large number of lines being enrolled and a sample of animals being followed from each line. The broad intent is to collect information from lines of sheep that experience higher mortality and lines that experience only background (low or zero) mortality. Since only 16-20% of lines may have higher mortality risk, it means that any representative sampling approach will sample far more low mortality lines than high mortality lines. Sampling requirements as outlined in Appendix 1 suggest that all lines would need to be sampled to ensure enrolment of sufficient lines of higher mortality sheep to achieve reasonable statistical power. While the requirement at the line-level is high, the requirement for sampling within each line is less onerous and sample size estimations indicate that enrolling a sample of 50 to 100 sheep from each line is likely to be sufficient. The design implication of this is that a field study program would need to provide the sort of detailed data collection as described in studies such as Norris et al (1989ab) or Makin et al (2008) but do so for all sheep that are exported over a period of between 2 to 3 years. This is likely to be very expensive and difficult to manage effectively. If a smaller scale approach is used (smaller sample size for example), the combination of low background mortalities and variable and unpredictable occurrence of mortality events means that the approach would be risky and not robust. For these reasons, this approach is not favoured by the authors.

An alternative approach which is favoured by the authors is to develop the two component capabilities as described in sections 9.1 and 8.2 of this report.

The first component (information management system or IMS) provides monitoring capacity across the entire industry that is capable of describing mortalities at the farm or line level in a sustainable fashion over multiple years. This approach will produce a large amount of relatively simple data allowing descriptions of patterns in mortality risk including patterns from year-to-year, by geographic distribution and at farm or PIC level. It is important to note that these data are unlikely to provide detailed information about risk factors that may be operating at the farm-level but they lay an important foundation that allows further research to be developed to address farm-level factors.

The second component (Experimental studies to investigate inappetence and inanition) provides experimental capacity that can allow detailed studies to be performed to investigate animal and line-level factors that influence appetite and morbidity under conditions similar to export assembly periods.

The combination of these two approaches provide capacity to describe and investigate risk factors for the two major causes of mortality (inanition and salmonellosis) no matter where those risk factors may be operating (animal, farm, truck, feedlot).

It is expected that the two component approaches will be used to inform each other. There are several ways that this is expected to occur:

- Information from the IMS may be used to inform design and conduct of experimental studies to investigate animal and line-level factors influencing outcomes of interest. Identification of lines or regions with elevated risk of inappetence/salmonellosis/mortality can be followed by approaches to identified farms to select sheep for inclusion in experimental trials under controlled conditions to investigate hypotheses concerning potential explanatory factors for the variability in risk.
- Results from animal-level studies performed under experimental conditions may also generate hypotheses about line-level risk factors that can in turn be investigated by collection of additional information from farms about management and other farm-level factors.
- Information from the IMS may also be used to inform design and conduct of nested studies involving additional data or samples being collected from specific lines of sheep and from their properties of origin to investigate hypotheses concerning line-level risk factors that may explain variability in outcomes of interest.
- Intervention studies may also be designed and implemented either on samples of sheep managed under routine export conditions or under experimental conditions to compare or assess potential interventions/treatments that are intended to reduce or prevent risk of morbidity or mortality in export sheep.

The ability to perform these studies would benefit from the presence of the integrated monitoring system to assist in collection of data on outcomes associated with health and welfare.

Development of more detailed terms of reference for studies that may be conducted under this section is considered to be dependent on results from analyses of data collected from a functioning IMS as described under section 8.1 and to a lesser extent on an existing capacity to perform experimental studies under controlled conditions as described under section 8.2. Detailed objectives and methodology are therefore not presented in this report.

9.4 Understanding and managing Salmonella exposure

9.4.1 Background and introduction

Section 8.3 describes development of the capacity to perform different experimental studies to investigate inappetence and inanition. Salmonellosis and inanition have been identified as the two major causes of mortality in export sheep in multiple studies over the past two decades. This section describes approaches intended to develop understanding of the factors influencing Salmonella exposure and to identify management measures that may be used to reduce exposure risk. More detailed information about salmonellosis in export sheep and recommendations for further assessing and managing salmonellosis, can be found in Makin et al (2008).

Several factors are important in the development of salmonellosis. These include Salmonella challenge, specific immunity to Salmonella infection, general state of health and resistance to infection . Ingestion is thought to be the most common route of infection though exposure may also occur via inhalation or conjunctival contamination.

Very few sheep are shedding Salmonella organisms when they enter an assembly depot. In some cases sheep may be expected to have higher risk of shedding and active infection on arrival for example sheep sourced from saleyards or dealer operations, sheep that have undergone frequent or prolonged transport movements, or that have suffered reduced feed access, or other stressors.

Even when most sheep are not shedding on arrival, over time in a system designed to accommodate heavy throughput of animals, the assembly feedlot will eventually be contaminated with Salmonella through organisms shed by clinically infected animals and passive shedders. Salmonella organisms can survive for months to years in the environment and under favourable environmental conditions organisms are capable of very rapid proliferation. The combination of small numbers of infected animals and favourable environmental conditions can lead to very high levels of organisms in the environment, heavy exposure of other sheep and when exposed sheep are susceptible to infection, this is when outbreaks of salmonellosis are considered likely to occur.

The expected pattern of disease associated with Salmonella infection in an individual assembly feedlot is for low incidence of disease very early in the feedlot period and as the feedlot period progresses, there is a higher likelihood of animals becoming infected and of increasing environmental contamination and more cases occurring towards the end of the assembly period. In some cases where there is sustained and heavy use of the assembly feedlot for multiple shipments (one after the other), the level of environmental contamination may build over time to a very high level and leading to rapid and high incidence of disease earlier in a feedlot assembly period.

This pattern is consistent with observations of low prevalence of Salmonella infections in sheep when export demand is low and assembly yards are either stocked at lower rates or are rested between shipments, and with increased risk of exposure and occasional spikes of salmonellosis that may occur under conditions of higher throughput. A variety of factors then influence this overall pattern and may increase or reduce the risk of salmonellosis including factors associated with farm-of-origin, transport, feedlot and extrinsic factors such as climate.

The outcome of the host-pathogen interaction at the individual animal level is variable and ranges from resistance to infection to acute, fulminant bacteraemia, endotoxaemia, and death. There are a large number of causal factors that may influence the outcome of host-pathogen interactions and many of these factors are likely to be capable of rapid and frequent change both within and between animals and lines. Healthy sheep have sufficient host resistance to be reasonably resistant to exposure to Salmonella. However, stressors such as transport, yarding, inappetence, concurrent disease and inclement weather can all decrease host resistance and increase susceptibility to Salmonella. While healthy sheep with no apparent predisposing conditions can still be infected with Salmonella and develop clinical disease, there appears to be a vast difference in susceptibility. Exposure doses as low as hundreds of Salmonella organisms may be sufficient to cause disease in compromised sheep while as many as 10^9 organisms may be required to cause disease and mortality in healthy sheep (Wray and Linklater 2000).

The growth of Salmonella in the rumen following ingestion is also influenced by dietary intake and gut factors in a relatively complex interplay of causal factors. Reduced feed intake is known to increase Salmonella numbers in the rumen and may also increase Salmonella virulence. Feeding after a period of starvation is also associated with multiplication of Salmonella as are dietary changes that result in clinical or subclinical ruminal acidosis.

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

Experimental Salmonella challenge trials provide an insight into the course of the disease. Following challenge animals become febrile and anorexic within 36 – 72 hours. The onset of disease is more rapid when the challenge dose is large, the host compromised or the virulence of the infecting strain is high. Diarrhoea is usually observed within 24 hours of the onset of fever. In most challenge experiments the majority of mortalities are observed between 3 to 10 days after challenge. Animals that survive 14 days following challenge are unlikely to die.

A separate section of this report deals with studies intended to test efficacy and safety of a vaccine against Salmonella. This section is focused on studies intended to lead to better understanding of exposure risk for Salmonella in export sheep and to identify potential interventions to reduce this risk.

Projects and activities include:

- Development of methods for measuring and monitoring levels of viable Salmonella organisms in assembly depot yards and paddocks as a measure of exposure risk.
- Assessment of interventions intended to reduce exposure risk of Salmonella in assembly feedlots including:
  - Alternating paddocks from one shipment to the next, scraping yards and spreading of lime
  - Treating paddocks or bedding (on-board ship) to manipulate virulence of Salmonella organisms to shift the population of environmental organisms to one that is dominated by Salmonella with low virulence
  - Shortened feedlot period
  - Investigation of the feasibility and impact of moving to a one-way flow system in assembly yard design that incorporates features such as separate arrival and load-out yards and separate areas for handling sick or compromised animals. Cost is likely to be a constraint for this option
- Assessment of interventions intended to reduce susceptibility to exposure and infection including:
  - Effect of minimising curfew of feed in sheep being transported to the assembly feedlot

9.4.2 Aim

To develop methods to assess Salmonella exposure risk in assembly feedlots (and other areas such as farm of origin and export ship) and subsequently use these to both describe exposure risk and assess interventions intended to reduce exposure risk in export sheep.

9.4.3 Objectives

1. Development of assays capable of measuring Salmonella organisms in the environment including:
   - Review options for development of an assay capable of measuring Salmonella organisms in assembly depot yards and paddocks as a measure of exposure risk and develop methods for assessing levels of environmental loading (exposure risk) and virulence
   - Development and validation of candidate assays under experimental conditions and under field conditions
2. Describe exposure risk for Salmonella in assembly feedlots using the methods developed under Objective 1 in observational studies in feedlots during routine voyages. Outcomes can be related to mortality in sheep lines using the approaches developed under section 8.1.
3. Review possible interventions intended to reduce Salmonella exposure risk and prioritise them based on cost and expected efficacy. Develop protocols to apply interventions under a field trial situation with untreated control groups for comparative purposes and assess exposure risk using methods developed in Objectives 1 and 2

9.4.4 Methods

The objectives outlined above are based largely on the findings of the recent report by Makin et al (2008) and represent a continuation of the work performed in that project. It is important to note that the IMS described in Section 8.1 of this report provides important functionality that will be of value in data collection and analysis for all studies of morbidity and mortality in export sheep including these. These studies are therefore intended to be developed following the development of the IMS described in Section 8.1.

The key objective in this section is Objective 1 and it is logical to break this section into a two-stage project with the first stage being proof of concept or initial development of a robust assay that is capable of measuring the level of Salmonella contamination in environmental samples and ideally the virulence.

Objectives 2 and 3 then represent applications of the assay method, firstly to describe exposure patterns in normal operations and secondly to assess various interventions intended to reduce or manage exposure risk.

9.5 Vaccination against Salmonella

9.5.1 Background and introduction

The terms of reference for the current report are focused on advancing knowledge and understanding concerning farm-level risk factors associated with mortality in export sheep and particularly mortality due to Salmonella and inanition. Salmonellosis and inanition have been identified as the two major causes of mortality in export sheep in multiple studies over the past two decades. Vaccination of sheep has been identified as a potential option for reducing mortality risk due to Salmonella infection.

A recently completed report (Perkins et al 2009) has provided a cost-benefit analysis on the feasibility of oral vaccination against salmonellosis in export sheep. The draft report has been submitted for consideration and industry feedback. The report indicates that development of an oral vaccination against Salmonella appears to be cost effective though it is dependent on assumptions concerning a number of factors and particularly exchange rates and sale price of sheep. However, the development of an oral Salmonella vaccine is considered to be of sufficient potential value to be worthy of further research into feasibility and efficacy and safety. Such work would be essential in further evaluating the potential of the vaccine to contribute to improved health and welfare of export sheep and in the event that a decision is made to register a product, the findings would be expected to contribute to a registration application package.

The report by Perkins et al (2009) identified a DNA Adenine methylase (DAM) attenuated Salmonella vaccine as the primary candidate for development and registration. Experimental challenge trials with DAM vaccines have been conducted in mice, poultry, and cattle (Heithoff et al 1999, 2001; Dueger et al 2001ab, 2003ab; Mohler et al 2006, 2008). These trials have produced similar results demonstrating the capacity for induction of rapid innate immunity within 24 hours of vaccination, induction of homologous and heterologous protection in each species, and absence of immunosuppression. Recently one of the authors (J House) has been involved in conducting preliminary trials in sheep. Previous studies in poultry and cattle have focused on oral vaccination of neonates. A potential limitation in adults is interference from the rumen flora following oral vaccination of adult livestock. Oral delivery of the vaccine with a dose of 10^7 was found to be effective at establishing the vaccine in adult sheep and was not associated with any adverse vaccine reactions or anorexia. The performance of this vaccine in other species suggests it may be well suited to application in the live sheep trade as it provides homologous and heterologous protection and could be delivered in drinking water. However, further trials in sheep would be required for APVMA registration.
Clinical trials in sheep would need to unequivocally establish safety and efficacy using the formulation, dose and route of administration as would be intended for the registered product, and with trials performed using animals of similar type to those in which the vaccine would be intended to be used. While previously completed trials provide very useful information, whether or not they can be used directly in a registration application may depend on whether the product used in the previous trials was identical to the product intended to be registered in Australia.

There are established protocols for experimental challenge trials that can be used to demonstrate efficacy of the vaccine against challenge with the types of Salmonella that the vaccine is intended to protect against.

Field trials involving efficacy and safety data collected on vaccinated and non-vaccinated animals under normal management conditions during live export may also provide supportive evidence in an application for registration. However, there may also be a case for claiming that an application for registration be based completely on experimental challenge trials and that field trials not be considered essential for registration. This is because there is considered to be a reasonable or high likelihood that field trials may return equivocal evidence of vaccine efficacy not because of failure of protection by the vaccine but because of unpredictable variability in the presence and severity of infectious challenge under natural conditions and the complex interaction of multiple different causal factors other than challenge with infectious agent that may influence the occurrence and severity of clinical disease due to Salmonella.

Project aims and objectives for studies intended to assess safety and efficacy of a Salmonella vaccine in export sheep and meet registration requirements for a vaccine to be registered for use in Australia. While not directly addressing current gaps in knowledge, these activities do provide an avenue for reducing the mortality impact of salmonellosis in export sheep.

Recent information from one of the authors (J. House, personal communication 2009) indicates that there may be treatments that can be applied to animals to enhance innate or non-specific immune mechanisms with protective effects against Salmonella challenge. It is suggested that consideration be given to assessing such treatments during clinical trials designed to test vaccine efficacy since the challenge trial approach provides a suitable design approach for testing efficacy.

9.5.2 Aim

To develop options for manufacture of an oral, attenuated Salmonella vaccine in Australia and undertake experimental and field challenge studies in sheep to test efficacy and safety of the vaccine against Salmonella challenge.

9.5.3 Objectives

1. Define requirements for registration of an oral, attenuated Salmonella vaccine in Australia and develop draft protocols for studies designed to achieve these requirements including:
   - Defining the vaccine product formulation
   - Summarising previous studies that have used the same (or similar) formulations in trials that may be relevant to registration of a veterinary vaccine including safety and efficacy trials.
   - Define requirements for an application package to be considered by the APVMA for registration of the vaccine for use in sheep in Australia.
2. Develop an agreement with a manufacturer capable of manufacturing vaccine to the formulation required and at a standard of quality assurance and good manufacturing practice that will meet or exceed Australian registration requirements.
3. Development of detailed study protocols in a staged process designed to meet or exceed requirements for submission of an application package to the APVMA for registration of the vaccine for use in sheep in Australia.
   - Experimental trials confirming safety and efficacy using the product formulation as intended for the registered product. Progress to following steps would be dependent on satisfactory results from these trials.

- Develop design modifications to include treatment groups as required to test treatments designed to enhance innate or non-specific immunity against Salmonella challenge.
- Completion of experimental challenge trials and field trials as required to support an application for registration.

4. Submit an application package to the APVMA for registration of the vaccine for use in Australian sheep.

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11 Appendix 1: Sample size estimation

Sample size estimations were performed using commercial software designed for power analysis and sample size estimation (PASS[d]).

A number of assumptions were made based on previous data relating to the Australian sheep export industry.

Background mortality rates were estimated to be 7 deaths per 1000 sheep based on the overall voyage mortality rate observed in B.LIV.0123.

B.LIV.0123 observed that 72% of all mortalities were associated with 16% of lines. It was assumed that there were 4 times as many lines available for sampling from background mortality lines compared with higher mortality lines.

Sample size estimations were based on comparisons of proportions (mortality expressed as a proportion) with statistical analyses based on relative risk estimates. Different relative risk (RR) estimates were used for generation of sample size, including 1.5, 3, 5 and 10. A RR=1.5 can be interpreted as indicating a risk factor that is associated with a 50% increase in the risk of mortality compared with the reference level, and a RR=5 is associated with a 5-fold increase in the risk of mortality compared to the reference level. Generally speaking a RR of 1.5 is at the lower limits of biological plausibility and may be consistent with more subtle or complex associations between the risk factor under study and the outcome. In contrast, where the RR is 3 or greater it indicates a very strong biological association.

Sheep are clustered into lines in the export industry and a statistical parameter called the intra-class correlation coefficient (ICC) can be used as a measure of the level of correlation between units (individual sheep) selected from the same cluster (line). Sample size estimations were performed using a range of ICC values including zero (indicating no clustering and used as a reference level for comparison, very low level of similarity or clustering (ICC=0.05), low (ICC=0.15) and moderate to high (ICC=0.25). The impact of clustering is to reduce the effective sample size meaning that the actual sample size must be increased to produce the same level of statistical power.

All sample size estimations used alpha=0.05 and beta=0.2. Statistical power is equal to 1-beta (0.8). If power=0.8, then the estimated sample size can be interpreted as the sample size required to achieve an 80% probability of obtaining a statistically significant result if the underlying association is significant.

Results are provided in Table 2. A probability of mortality in the background lines of 0.007 is equivalent to 7 deaths per 1,000 sheep. Risk factors that are subtle or more complex may be associated with a lower RR value and generally require a higher sample size to achieve the same power. The largest sample size is required for those scenarios where the RR is lower (1.5). The impact of clustering is interesting. Where there is no clustering, there is no change in the total sheep numbers required when the scenario changes from sampling 50 sheep per line to 100 sheep per line. However, as the effect of clustering gets more pronounced (ICC larger), the most efficient sampling scheme is one that samples fewer sheep per line and more lines.

The impact of increasing the sample per cluster is also interesting. If the sample size per cluster is increased from 100 to 200, there is relatively little impact on the number of clusters that need to be sampled except where the ICC=0. When ICC=0, the total number of sheep to be sampled remains the same so the number of clusters is reduced by 50% when the sample size per cluster

[d] http://ncss.com

is increased from 100 to 200. However, when ICC is greater than zero this effect is much reduced. The impact is generally a very small reduction in the number of clusters that must be sampled but a substantial increase in the total number of sheep sampled since the number of sheep per cluster is doubled. There is therefore little evidence of any benefit of sampling more than 100 sheep per cluster and reasonable evidence to support a cluster-level sample size of 50 sheep.

The scenarios that are considered most relevant are those that involve RR values of 1.5 or 3, and ICC values of 0.15 or 0.25. These reflect associations that are relatively subtle and allow for low to moderate effects of clustering. More powerful associations would be detected as well with these sample sizes. The results indicate a requirement for sampling from 1,050 to 1,750 lines of higher mortality sheep and more than 4,000 lines of sheep experiencing background mortality.

To put these requirements into context, B.LIV.0123 collected data on 27 voyages over a period of just under three years and in that time were able to collect data on a total of 10,428 lines of sheep. If we assume that 20% of these lines were higher mortality lines then we would expect 2,085 higher mortality lines. Therefore a study that aims to collect data from as many as 1,750 lines of sheep that experience higher mortality, will need to be conducted over a large number of voyages (around 23 to 25 voyages) and will therefore require collection of data from all eligible voyages over a period of about three years. If the study were to collect data from ~1,000 lines of sheep that experience higher mortality, it would need to be conducted over about two years.

Table 2: Sample size of clusters (lines) and sheep required **to achieve power of 0.8, given varying assumptions concerning probability of death in background lines and relative risk**

	Clusters (lines)		Sheep sampled	Total	ICC	Probability of death		Relative
Clustering	Background	Hi risk	per line	sheep		Background	Hi risk	risk
NONE	528	132	50	33,000	0	0.007	0.0105	1.5
ICC=0	264	66	100	33,000	0	0.007	0.0105	1.5
	48	12	50	3,000	0	0.007	0.021	3
	24	6	100	3,000	0	0.007	0.021	3
	20	5	50	1,250	0	0.007	0.035	5
	12	3	100	1,500	0	0.007	0.035	5
Very low	1,820	455	50	113,750	0.05	0.007	0.0105	1.5
ICC=0.05	1,572	393	100	196,500	0.05	0.007	0.0105	1.5
	168	42	50	10,500	0.05	0.007	0.021	3
	144	36	100	18,000	0.05	0.007	0.021	3
	60	15	50	3,750	0.05	0.007	0.035	5
	52	13	100	6,500	0.05	0.007	0.035	5
	20	5	50	1,250	0.05	0.007	0.07	10
Low	4,408	1,102	50	275,500	0.15	0.007	0.0105	1.5
ICC=0.15	4,184	1,046	100	523,000	0.15	0.007	0.0105	1.5
	400	100	50	25,000	0.15	0.007	0.021	3
	380	95	100	47,500	0.15	0.007	0.021	3
	140	35	50	8,750	0.15	0.007	0.035	5
	132	33	100	16,500	0.15	0.007	0.035	5
	48	12	50	3,000	0.15	0.007	0.07	10
Moderate	6,992	1,748	50	437,000	0.25	0.007	0.0105	1.5
ICC=0.25	6,792	1,698	100	849,000	0.25	0.007	0.0105	1.5
	632	158	50	39,500	0.25	0.007	0.021	3
	616	154	100	77,000	0.25	0.007	0.021	3
	220	55	50	13,750	0.25	0.007	0.035	5
	216	54	100	27,000	0.25	0.007	0.035	5
	76	19	50	4,750	0.25	0.007	0.07	10