final report

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Prepared by: Kate Makin

John House
Nigel Perkins
Greg Curran
The University of Sydney
AusVet Animal Health Services
NSW DPI

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Abstract

The objective of the LIVE.123 project was to determine the rate, causes and predisposing factors of mortality for live export sheep as well as the relative mortality risk for different lines of sheep. Approximately 1.65 million sheep in 24 shipments were tracked from farm of origin to port of discharge between September 2005 and June 2008. Salmonella induced enteritis was the most common cause of mortality (34.4%), followed by inanition (23.9%) and enteritis/inanition (18.2%). Seventy-four percent of mortality was traced to 18% of lines. Pastoral sheep from NSW and Queensland sheep were found to have higher voyage mortality than other sheep. Other risk factors for mortality were age, ship and time of year. Factors identified as important drivers of disease were level of salmonella exposure and host immunity. The observation that specific lines of sheep from certain locations are more likely to die suggests that the immunity of sheep from these locations is compromised by property of origin factors or by the process involved in getting these animals to the assembly depot. Recommendations for reducing mortality include minimising salmonella challenge at the assembly depots, promoting consistent industry adoption of the road transport guidelines, avoid sourcing sheep from high risk locations pending clarification and correction of the risk posed to these sheep, implementation of a uniform information management system across industry to track sheep performance from origin to discharge, and improve the training and definition of responsibilities for shipboard veterinarians and stockmen.

Executive summary

The objective of the LIVE.123 project was to determine the rate, causes and predisposing factors of mortality for live export sheep and lambs as well as the relative mortality risk for sheep and lambs by region and time of year, including whether or not pastoral sheep and lambs were more at risk for mortalities. The project also aimed to investigate the relationships between inappetence and salmonellosis incidence and formulate additional strategies for producers and exporters that can be applied prior to arrival at the pre-export assembly depot to improve sheep health.

Data was collected for 39 voyage assemblies between September 2005 and June 2008. Assembly depot mortality was low (5 deaths/10,000 sheep) with the exception of the assembly for voyage 22 (55 deaths/10,000 sheep) that experienced an outbreak of salmonellosis. Mortality on receival was largely due to trauma, other sporadic causes of mortality occurred throughout the assembly period while salmonellosis was the main cause of death during the later part of the assembly period.

Shipboard mortality data was collected from 27 voyages between September 2005 and June 2008. Three of these voyages were excluded from the data analysis as less than 50% of the sheep that died could be traced to property of origin. The overall traceable mortality for the remaining 24 voyages was 69.87%. Mortality was restricted to certain lines of sheep with 62.7% of lines having no recorded mortality and 74% of mortality being traced to 18% of lines. Sheep from the NSW pastoral zone were found to have higher voyage mortality than other sheep, this was heavily influenced by two lines of pastoral sheep that had high mortality. Queensland sheep also had higher mortality. Distance travelled to the assembly depot was not the driving risk factor for mortality as sheep that travelled more than 800km to reach the assembly depot did not have a greater risk of mortality than sheep that travelled less than 200 km. The Queensland sheep were included in consignments originating from Victoria. In addition to this, mortality was highest in the second half of the year, old sheep and ewes had a higher mortality risk than young sheep and lambs and certain ships had a higher mortality risk. Enteritis and inanition continue to contribute significantly to mortality in sheep exported live by sea, accounting for over 76% of diagnosed mortality. Enteritis was found to be the most common cause of mortality (34.4%), followed by inanition (23.9%) and enteritis/inanition (18.2%). Heat stress was recorded as the cause of death for 9.5% of diagnosed mortality however, heat stress deaths were largely confined to two voyages that had heat stress events.

Salmonellosis and inanition remain the most common causes of mortality in the live sheep trade. Over the past 10 years, the demographic of the exported flock has changed with more young sheep and fewer older, heavy wethers entering the trade. Because of this, inappetence and negative energy balance may no longer be the primary drivers of disease and mortality. Factors identified as important drivers of disease in the live sheep trade were level of salmonella exposure and host immunity. It is difficult to determine which disease processes are occurring first as the biological pathways are complex and the factors that affect the development of disease are continually fluctuating.

Recommendations for reducing mortality include minimising salmonella challenge at the assembly depots through regular cleaning/scraping of sheep yards and avoiding mixing of “carry over” sheep into newly introduced mobs. The finding of increased risk of death associated with curfew times in excess of those stipulated by the road transport guidelines indicates a need to promote industry compliance. Implementation of a standardised stock tracking system across the industry to trace sheep performance from property of origin to discharge from ship would facilitate problem solving

and continued advancement of management practices. A prototype for such a system has been developed as part of this project.

Ship board veterinarians are currently under utilised. The training and definition of roles and responsibilities for shipboard veterinarians and stockmen should be reviewed to improve sheep management and problem solving capacity.

Future research and development should investigate why sheep sourced from the high risk areas identified in this study are more likely to die. Generic investigation of risk factors for mortality is difficult due to the low and variable incidence of mortality. The finding that sheep from specific locations were more likely to die provides an opportunity for targeted investigation of disease risk factors and a means to evaluate the effectiveness of potential interventions.

The advent of electronic identification in the form of NLIS ear tags provides a refined means of investigating the behaviour of sheep as it relates to proximity to feed and water troughs to address the question of feeding behaviour and how it can be manipulated.

During the last 10 years there has been a significant development in the area of salmonella vaccines with the advent of DNA adenine methylase live attenuated vaccines. These vaccines may offer a cost effective means of reducing salmonellosis in the live sheep trade.

In regards to the management of disease outbreaks, the current approach is inconsistent, lacks a scientific basis, appears suboptimal, and leaves the industry prone to criticism for not having a robust plan to manage the most common cause of mortality. Research is required to evaluate existing and alternate disease management options.

The conclusions and recommendations of the LIVE.123 project are aimed at improving sheep health and welfare through clarifying the mortality risk for sheep of different classes and from different sources, by identifying the most common causes of mortality and through provision of a tool for monitoring and investigating sheep performance.

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1 Background

Although extensive mortality investigations were carried out in West Australia (WA) in the 1980s and early 1990s (Richards et al 1990, Richards et al 1991, Norris et al 1990, Higgs et al 1991, Higgs et al 1993) and to a lesser extent in Victoria (Kelly 1996) in the same time period, causes of mortality in the live sheep trade remain incompletely understood, in particular those factors at farm of origin, during road transport, during the assembly period and on ship that predispose to mortality. During 2001 and 2002 higher mortality was observed in sheep exported from the eastern states. This mortality was anecdotally reported to be associated with an increase in the incidence of salmonellosis during the assembly period. These reports raised questions regarding the association between inappetence and salmonellosis and how it may be prevented. In 2005 one of the risk management strategies included in the live export standards was the placement of restrictions on the export of pastoral sheep, sheep originating more than 800 km from the pre-assembly depots and all lambs during the May to October period. These restrictions appear to have been based on anecdotal reports that these classes suffer higher mortalities than other sheep at that time of the year. No scientific evidence was available to support these restrictions, in fact previous research conducted in West Australia (Higgs et al 1999) suggested that mortality was lower in sheep that travelled further to the assembly depots. The LIVE.123 Project was initiated to provide information on causes of death in live export sheep, to identify factors contributing to the risk of death, and to determine if the risk of mortality for pastoral sheep and lambs during the May to October period was higher than that for other classes of sheep.

2 Project objectives

1. Determine the rate, causes and predisposing factors of mortality for live export sheep and lambs at the different stages of the live export supply chain (to the port of discharge).

2. Determine the relative mortality risk for sheep and lambs by region and time of year, including whether or not pastoral sheep and lambs are more at risk for mortalities than sheep and lambs from non-pastoral areas.

3. Determine the relationships between inappetence and salmonellosis incidence at the pre-export assembly depot and on ship as related to on-farm, transport, seasonal and regional factors.

4. Formulate additional strategies for producers and exporters that can be applied prior to arrival at the pre-export assembly depot to minimise the level of inappetence, salmonellosis and other conditions that affect the health of live export sheep and lambs delivered to Portland and Adelaide, as well as sheep exported from Perth in split shipments.

5. Develop a working prototype computerised information management system to record health and mortality data on sheep during all stages of live export and formulate a detailed project proposal for development of a final working version of this system.

3 Project overview

The overall project has incorporated several component projects involving collection of samples and observational data on mortalities from assembly period through the voyage to the point of discharge, as well as incorporation of data derived from industry records. Data associated with 39 voyages were collected between September 2005 and June 2008. This overview section attempts to provide a brief description of each of the component activities to ensure that they can be placed in context of the overall project.

3.1 Definitions

Line A group of sheep of a single class, sourced from a single vendor intended for a single assembly period and subsequent voyage.

Consignment A group of sheep, sourced for multiple vendors and assembled at a single port of loading for a single export voyage

Research Lines Special lines of sheep included in consignments as part of the LIVE.123 project. Sourcing these lines for export would have otherwise been prohibited between May and October under the Australian Standards for the Export of Livestock.

These groups were: 1) sheep from the pastoral zone (North of Port Augusta in South Australia and from the Western districts in NSW, 2) sheep travelling more than 800km to reach an assembly depot, and 3) lambs.

3.2 Project components

3.2.1 Detailed assembly period investigations

3.2.1.1 Observational data

Members of the project team were present at the assembly depot during the assembly period for 11 voyages, comprising 18 assembly period events. Multiple assembly periods events are possible for a single voyage reflecting the fact that sheep are often assembled at more than one assembly depot. The assembly period for each assembly depot is considered as a distinct entity as the conditions in the assembly depots can be very different reflecting the location and husbandry practices in each of the assembly depots. Team members were able to observe sheep arriving at the assembly depot (end of the journey from property of origin), and collect receival and assembly period data. There are two assembly depots in Portland (Victoria) and Adelaide (South Australia), both assembly depots in each location often provide sheep to a single voyage and team members were able to collect similar data from both assembly depots during the time when they were assembling sheep for the same voyage.

3.2.1.2 Intensive investigations

Members of the project team carried out cause of mortality investigations during 3 assembly periods, 1 in Portland, 1 in Adelaide and 1 in Fremantle. Cause of death was investigated by performing postmortem examinations and collecting samples for diagnostic testing. Blood, faecal and environmental sampling was carried out during 1 assembly period in Portland and 1 assembly period in Adelaide. In Adelaide, additional environmental sampling was carried out during 5 assembly periods and at 3 time points when no sheep were assembled.

3.2.2 Detailed voyage investigations

Members of the project team accompanied 5 voyages. During the voyage team members collected mortality data and investigated causes of mortality. Cause of mortality was investigated by performing postmortem examinations and collecting swabs and tissue samples for diagnostic testing.

3.2.3 Industry sourced data

3.2.3.1 Assembly period

A quantity of industry sourced data was used for all voyages studied, including those for which project member(s) were present. In addition to the 11 voyage assemblies attended by members of the project team, receival data were collected for a further 28 voyages. For these 28 voyages assembly data were provided by industry sources.

3.2.3.2 Voyage

For voyages that were not accompanied by a member of the project team, data were sourced from AQIS Accredited Veterinarians (Livestock). Mortality data were sourced for 22 unaccompanied voyages and postmortem records were available for 14 of these 22 voyages.

3.2.4 Data management and analysis

Morbidity and mortality data from different sources and shipments were aggregated to form combined datasets covering assembly period and voyage. Data relating to salmonella culture and serum concentrations of Non Esterified Fatty Acids (NEFA), were managed as separate datasets. Data were analysed initially using univariable and bivariable techniques to produce summary statistics and to screen possible explanatory variables of interest. A variety of multivariable analytical techniques were then used to investigate associations between multiple explanatory variables and outcomes of interest. These included analysis of variance (ANOVA), poisson analysis, logistic regression and survival analysis. Selection of a particular analytical approach was determined primarily by the structure and format of the data and the objectives of a particular analysis.

3.2.5 Development of an information management system

An electronic information management system (IMS) was developed during the project. The IMS was developed to facilitate collection of data of value to the project and to provide a valuable on-going resource to industry operators. The IMS was designed as a tool to allow operators to efficiently and accurately manage data, fulfil reporting obligations (both regulatory and business) and to facilitate ongoing quality assurance and early identification of problems.

3.3 Summary of components

Table 3.1 outlines which of the project components were utilised for each of the 39 shipments investigated. For some shipments both assembly period and voyage information was available. For other shipments only assembly period information was available.

Table 3-1 Summary of the project components utilised in the investigations of the 39 voyages studied in the project.

	Assembly Period			Voyage	
	Team Member(s) Present		Industry Data	Team Member(s) Present	Industry Data
Voyage Code	Observational Data	Sampling & Postmortems			
1	✓	✓	✓	✓	✓
2	-	-	✓	-	✓
3	-	-	✓	-	✓
4	-	-	✓	✓	✓
5	✓	✓	✓	-	✓
6		-	✓	-	✓
7		-	✓	-	✓
8		-	✓	-	✓
9		✓	✓	✓	✓
10		-	✓	-	✓
11		-	✓	-	✓
12		-	✓	-	✓
13		-	✓	-	✓
14		-	✓	-	✓
15	✓	✓	✓	-	✓
16			✓	-	✓
17	✓	✓	✓	✓	✓
18	✓	-	✓	-	✓
19		-	✓	-	✓
20		-	✓	-	✓
21	✓	-	✓	-	✓
22	✓	✓	✓	✓	✓
23			✓	-	✓
24	✓	✓	✓	-	✓
25		✓	✓	-	✓
26	✓	✓	✓	-	-
27		-	✓	-	-
28		-	✓	-	-
29		-	✓	-	-
30		-	✓	-	-
31		-	✓	-	-
32		-	✓	-	-
33		-	✓	-	-
34		-	✓	-	-

	Assembly Period			Voyage	
	Team Member(s) Present		Industry Data	Team Member(s) Present	Industry Data
Voyage Code	Observational Data	Sampling & Postmortems			
35		-	✓	-	-
36		-	✓	-	-
37		-	✓	-	-
38	✓	-	✓	-	✓
39	✓	-	✓	-	✓

4 Summary of voyages

Table 4.1 summarises all voyages studied during the LIVE.123 project. In total data was collected for 39 voyages, 9 of which included research lines. Year and month of receival is shown along with count of sheep accepted at the assembly depot and totals for each voyage and assembly depot. The voyage numbers used throughout the report are consistent with the numbers in Table 4.1.

Table 4-1 Summary of voyages studied in the LIVE.123 project including the count of sheep received at each assembly depot by voyage and identification of voyages with research lines. Table continued over page.

			Assembly Depot									Research
Year	Month	Voyage	1	2	3	4	5	6	7	8	Total	Lines
2005	September	1	20,687	21,067			60,409	3,760			105,923	✓
	October	2	30,798	24,589			19,579				74,966	
	December	26	23,782	40,265							64,047	
2006	January	31		40,948							40,948	
	March	3	29,115	31,282			11,788	796			72,981	
		5			36,712	38,186					74,898	
	April	4	27,751	27,137			45,773				100,661	
	May	6	15,839	16,826			40,282	6,410			79,357	
	June	7	28,357	5,250			70,171		2,054		105,832	
		27	20,535	26,118							46,653	
	July	8					43,060	12,151			55,211	
	August	9	17,519	17,077	12,620	25,844					73,060	✓
		10	12,060	22,110			41,651		326		76,147	
	September	28	34,209	26,132							60,341	
	October	11			41,369	35,630					76,999	
		12			36,914	34,388					71,302	
		29	21,535	21,908							43,443	

			Assembly Depot									Research
Year	Month	Voyage	1	2	3	4	5	6	7	8	Total	Lines
	November	30	30,414	28,403							58,817	
	December	13					55,524	227	80		55,831	
2007	January	32	37,045								37,045	
	May	15			15,481	24,793	41,507				81,781	✓
		33	36,596	9,736							46,332	
	June	16		18,508			33,268	11,613			63,389	
		17				28,362	58,078				86,440	✓
	July	18			14,493		23,451	39,532	7,926	1,738	87,140	✓
		19	26,337				58,456		204		84,997	
	August	20					69,541				69,541	✓
		21		33,627	40,848	28,621					103,096	✓
	September	22			41,402	28,468		45,058			114,928	
		23	28,830	28,358			71,372				128,560	
		34	27,889	28,086							55,975	
	October	24			45,455	31,676					77,131	
	November	25					72,891	864	5,234		78,989	
		35	37,861								37,861	
	December	36	10,993								10,993	
		37			39,279	29,554					68,833	
2008	February	14	49,254				22,648				71,902	
	May	38			27,606	24,776					52,382	✓
	June	39				18,463					18,463	✓
		Total	**567,406**	**467,427**	**352,179**	**348,761**	**839,449**	**120,411**	**15,824**	**1,738**	**2,713,195**	

5 Rejection and mortality on arrival at the assembly depot

5.1 Materials and methods

5.1.1 Data collection

Data were collected for 39 voyage assemblies and are summarised below in table 5.1. In addition to the data shown in table 5.1, the following information was recorded (where available) for each line of sheep received:

- Sheep information - age, breed, sex, average weight of sheep, as well as sheep dead on arrival and those rejected from the consignment.
- Truck information – net, gross and tare weights, registration, transport company and place of origin.
- Vendor information - name, location of property, property identification code (PIC), agent used and method of identification of sheep (e.g. ear tags, wool brands).
- Additional National Vendor Declaration (NVD) information – departure and arrival time and date as well as curfew time. The curfew time specified by producers on the NVD does not specify the time stock were held off feed verses water.

Additional data sourced for each consignment included distance travelled to the assembly depot, climate information for the property of origin and climate information for the assembly depot. For each consignment the property identification code was used to determine the nearest town to the property and distance, and climate information was sourced for this location. Distance travelled to assembly depot by road was calculated using web based distance calculators (http://code.google.com/apis/maps/documentation/services.html and http://www.travelmate.com.au) and climate information was obtained from the Australian Bureau of Meteorology.

Table 5-1 Incidence (per 10,000 sheep) of deaths (DOA) and rejection on arrival by voyage. Note, assembly data was not available for every feedlot for every voyage.

Assembly	Sheep			95% CI				95% CI	
Period Code	Received	DOA	Incidence	Upper	Lower	Rejects	Incidence	Upper	Lower
1	41,754	38	9.10	12.49	6.63	234	56.04	63.67	49.32
2	55,387	30	5.42	7.73	3.79	220	39.72	45.31	34.82
3	60,397	32	5.30	7.48	3.75	423	70.04	77.01	63.69
4	54,888	33	6.01	8.44	4.28	255	46.46	52.50	41.11
5	74,898	24	3.20	4.77	2.15	170	22.70	26.37	19.54
6	32,665	21	6.43	9.83	4.21	243	74.39	84.31	65.63
7	33,607	29	8.63	12.39	6.01	202	60.11	68.95	52.39
9	73,060	40	5.47	7.45	4.02	304	41.61	46.55	37.20
10	34,170	10	2.93	5.39	1.59	131	38.34	45.47	32.32
11	76,973	63	8.18	10.47	6.40	208	27.02	30.95	23.59
12	71,302	80	11.22	13.96	9.02	791	110.94	118.89	103.51
14	49,254	16	3.25	5.28	2.00	276	56.04	63.02	49.82
15	40,274	14	3.48	5.83	2.07	132	32.78	38.85	27.65
16	18,508	6	3.24	7.07	1.49	43	23.23	31.28	17.25
17	28,362	40	14.10	19.20	10.36	102	35.96	43.63	29.64
18	14,493	1	0.69	3.91	0.12	12	8.28	14.47	4.74
19	26,337	13	4.94	8.44	2.88	65	24.68	31.44	19.37
21	103,096	77	7.47	9.33	5.98	405	39.28	43.29	35.65
22	69,870	42	6.01	8.12	4.45	388	55.53	61.32	50.29
23	57,188	37	6.47	8.92	4.69	245	42.84	48.54	37.81
24	77,131	76	9.85	12.33	7.87	602	78.05	84.51	72.08
26	64,047	36	5.62	7.78	4.06	272	42.47	47.81	37.72
27	46,653	21	4.50	6.88	2.94	188	40.30	46.47	34.94
28	60,341	38	6.30	8.64	4.59	311	51.54	57.58	46.13
29	43,443	24	5.52	8.22	3.71	143	32.92	38.76	27.95
30	58,817	30	5.10	7.28	3.57	381	64.78	71.59	58.61
31	40,948	30	7.33	10.46	5.13	111	27.11	32.63	22.52
32	37,045	53	14.31	18.71	10.94	157	42.38	49.53	36.26
33	46,332	17	3.67	5.88	2.29	179	38.63	44.71	33.38

Assembly Period Code	Sheep Received	DOA	Incidence	95% CI Upper	95% CI Lower	Rejects	Incidence	95% CI Upper	95% CI Lower
34	55,975	33	5.90	8.28	4.20	183	32.69	37.78	28.29
35	37,861	21	5.55	8.48	3.63	160	42.26	49.32	36.21
36	10,993	4	3.64	9.35	1.42	96	87.33	106.52	71.57
37	68,833	75	10.90	13.65	8.69	471	68.43	74.87	62.54
38	52,382	28	5.35	7.72	3.70	171	32.64	37.91	28.11
39	18,463	3	1.62	4.78	0.55	52	28.16	36.91	21.49
Total	**1,735,747**	**1135**	**6.54**	**6.93**	**6.17**	**8326**	**47.97**	**49.01**	**46.95**

Table 5.1 shows the number of sheep received as well as incidence of death and rejection on arrival with 95% confidence intervals.

The overall incidence of death on arrival was 6.54 deaths per 10,000 sheep.

The overall incidence of rejection on arrival was 47.97 rejects per 10,000 sheep.

5.1.2 Statistical analysis

Analysis of mortality and rejection on arrival at the pre-export assembly depot was restricted to eastern (Portland and Adelaide) assembly periods involving four assembly depots. This restricted the statistical analysis to assembly data from 35 of the 39 voyage assemblies. Data collected from the West Australian depots was not included in this analysis as it was often not available and the drafting of rejects is managed differently in West Australia so the raw data are not directly comparable. Death on arrival represents deaths occurring during road transportation to the pre-export assembly depot. Rejection on arrival represents sheep rejected from the consignment at the time of unloading at the pre-export assembly depot; it does not include sheep rejected during classing or later in the assembly period.

The final data set contained a row for each line of sheep received. Body weight, curfew time and weather data for the assembly depot and property of origin were stored and analysed on a continuous scale. Distance travelled to assembly depot and class of sheep were classified into categories and are shown in table 5.3 below. Additional categorical variables included port, state of origin, year, month of year and assembly depot.

Negative binomial regression was used to analyse both death on arrival and rejection. Negative binomial regression is a special form of logistic regression for count data that are heavily clustered. The output of negative binomial regression is a relative risk. Relative risks are used to compare incidence rates; if the p-value is less than 0.05 then the difference between groups is significant.

5.2 Results

5.2.1 Summary statistics

Figure 5.1 shows the number and class of sheep received by each assembly depot. The figure shows that the predominant class of sheep were wethers (72%) followed by young wethers (23%) while pastoral sheep accounted for less than 2.5% of sheep studied. The pastoral sheep included wethers, young wethers, and rams. Assembly depots 1 and 2 are based in Portland and depots 3 and 4 in Adelaide. Figure 5.1 illustrates that the majority of pastoral sheep were assembled and loaded in Adelaide.

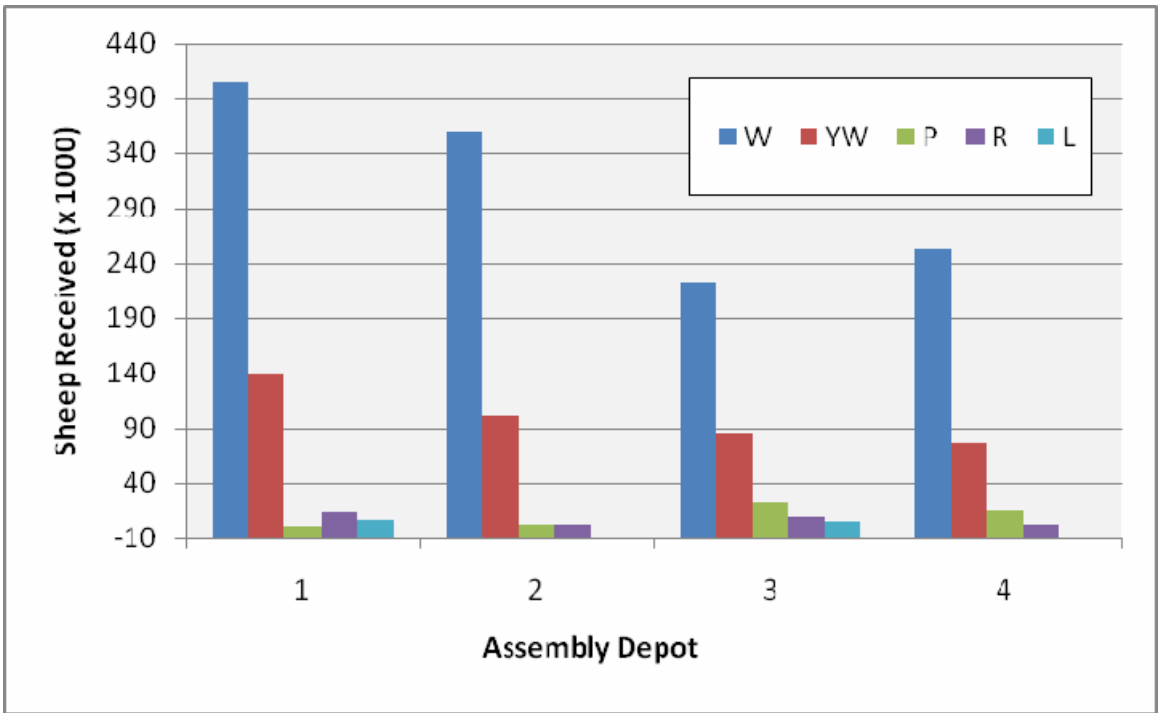


Figure 5-1 Total sheep received (in thousands) by class for each of the 4 eastern assembly depots studied (W=wethers, YW=young wethers, P=pastoral sheep, R=rams; L=lambs).

5.2.2 Multivariate analysis

5.2.2.1 Death on arrival

The final data set for each line of sheep received included continuous variables body weight, curfew time and weather data for the assembly depot and property of origin. Categorical variables included distance travelled to assembly depot, class of sheep, port, state of origin, year, month of year and assembly depot. Table 5.2 shows the results of the final multivariable negative binomial regression for death on arrival. Month was a significant variable in the univariate analysis and interactions were observed between month and other variables. As there were no consistent seasonal patterns month was added to the model as a random effect which improved the model fit.

Table 5-2 Multivariable negative binomial regression model for death on arrival (n = number of sheep, RR = relative risk, CI = confidence interval

					95% CI		
Variable	**Category**	**n**	**Deaths**	**RR**	**Lower**	**Upper**	**p-value**
Class	Young Wether	404,590	120	*Reference*			
	Wether	1,245,036	933	1.84	1.34	2.54	<0.001
	Ram	30,174	30	2.14	1.10	4.19	0.026
	Pastoral	41,802	40	1.98	1.01	3.89	0.047
	Lamb	13,666	7	1.72	0.41	7.19	0.454
Distance	<200 km	606,569	198	*Reference*			
	200-299 km	379,074	268	1.34	1.01	1.78	0.041
	300-499 km	314,089	177	1.27	0.94	1.73	0.124
	500-799 km	348,173	332	2.07	1.53	2.79	<0.001
	>= 800 km	87,842	160	3.38	2.29	5.00	<0.001
Journey Rejects[a]	No	1,226,739	543	*Reference*			
	Yes	509,008	592	1.99	1.62	2.44	<0.001
Body Weight	Kg			1.04	1.02	1.05	<0.001
Curfew	Hrs			1.00	0.99	1.01	0.568

[a] In regards to Journey rejects “No” indicates the number of sheep that were unloaded from trucks where there were no sheep rejected on arrival at the assembly depot. “Yes” indicates the number of sheep that were unloaded from trucks that included rejected sheep. This is not the number of sheep rejected rather the number of sheep delivered on trucks that included sheep that were rejected on arrival.

Compared to young wethers, the relative risk of mortality during road transportation was 1.84 times higher for wethers, 2.14 times higher for rams and 1.98 times higher for sheep from the pastoral zone. Distance travelled to the assembly depot also had a significant effect on risk of mortality. Compared to sheep in category 1 (travelling less then 200km), the relative risk of mortality was twice as high (2.07 times) for sheep in category 4 (500-799km) and more than three times higher (3.38) for sheep in category 5 (>800km). There was also an association between death during transport and transport injury (journey rejection). The relative risk of mortality, in receivals with journey associated rejection was twice as high (1.99 times) as that in receivals without journey associated rejection. Increasing body weight was significantly associated with mortality, for each 1kg increase in body weight, the risk of mortality increases by 1%. Curfew time did not impact significantly on mortality during road transportation. All other variables in the dataset were analysed and were shown to have no significant effect on the risk of mortality during road transportation.

5.2.2.2 Rejection on arrival

The variables analysed in regards to death on arrival were also analysed to investigate risks for rejection of sheep at arrival to assembly depots. Table 5.3 shows the results of the final multivariable negative binomial regression for rejection on arrival. Assembly period was a significant variable in the univariate analysis and interactions were observed between assembly period and other variables. As there were no consistent assembly period pattern assembly period was added to the model as a random effect which improved the model fit.

Assembly depot 4 does not have a weigh bridge to determine sheep weight. The data from the 4 assembly depots was analysed with and without body weight included in the analysis. The model presented provided the best fit and excluding sheep from assembly 4 as they did not have a recorded body weight did not appreciably change the relative risk of other variables or their significance.

Table 5-3 Multivariable negative binomial regression model for rejection on arrival.

(n = number of sheep, RR = relative risk, CI = confidence interval)

					95% CI		
Variable	**Category**	**N**	**Rejection**	**RR**	**Lower**	**Upper**	**p-value**
Assembly Depot	1	564,593	2,492	*Reference*			
	2	464,779	2,380	1.15	1.04	1.28	0.010
	3	350,305	1,572	0.59	0.49	0.71	<0.001
Distance	<200 km	603,875	198	*Reference*			
	200-299 km	377,051	268	1.25	1.11	1.41	<0.001
	300-499 km	312,388	177	1.30	1.14	1.47	<0.001
	500-799 km	345,750	332	1.87	1.60	2.17	<0.001
	>= 800 km	87,222	160	1.69	1.31	2.17	<0.001
DOA	0	1,521,745	6,666	*Reference*			
	>=1	204,541	1,660	1.69	1.49	1.92	<0.001
Average Body Weight	(kg)			1.01	1.00	1.02	0.040
Curfew	(hrs)			1.00	0.99	1.00	0.324

There was significant variation in incidence of rejection between assembly depots. This variation is likely due to management factors with some operators rejecting heavily on arrival and others rejecting heavily later in the assembly period. Distance travelled to the assembly depot also had a significant effect on risk of rejection. Compared to sheep in category 1 (travelling less then 200km), the relative risk of rejection was higher for all other groups. As was seen in the mortality model, there was an association between death during transport and rejection. The relative risk of rejection in

receivals with mortality during transport was 1.69 times higher than that in receivals without transport mortality. Increasing body weight was significantly associated with rejection, for each 1kg increase in body weight, the risk of rejection increases by 1%. Curfew time did not impact significantly on the risk of rejection. All other variables in the dataset were analysed and were shown to have no significant effect on the risk of rejection.

6 Assembly period mortality

Assembly period mortality is generally low in the absence of an outbreak of salmonellosis. During the 3 years of investigation, one outbreak of assembly period salmonellosis was studied. This occurred during the assembly period for voyage 22 at assembly depot 6 in West Australia. A report for this event is presented separately in section 6.4 below. Assembly period mortality for the eastern ports is presented below in sections 6.1 to 6.3.

6.1 Materials and methods

6.1.1 Data collection

During the pre-export assembly period mortality data (mortality count and day) were obtained from the official mortality records for each assembly depot.

6.1.2 Postmortem examinations

Post-mortem examinations were performed on sheep that died during the assembly period for voyages 1, 5 and 26. Postmortem findings were recorded using digital photography and samples were taken for diagnostic testing. Formalin fixed samples were collected for histopathology and tissue swabs were collected for salmonella culture. Cause of mortality was classified according to the criteria outlined in appendix 1.

6.2 Results

6.2.1 Mortality rates

Tables 6.1 and 6.2 summarise assembly period mortality data recorded for Portland and Adelaide. The tables show that the highest recorded assembly period mortality in the eastern assembly depots was 0.19% or 19 deaths in every 10,000 sheep. Overall assembly period mortality was 0.05% or 5 deaths per 10,000 sheep. In regards to terminology the day that sheep arrive at the assembly depot is referred to as the day of “receivals”. Assembly day refers to the number of days the sheep have been in the assembly depot since the day of receivals. This reference to time in the assembly depots generally works well in the eastern assembly depots as sheep tend to be loaded into each assembly depot in one day. The scenario is different in West Australia where sheep are often received over multiple days.

Table 6-1 Assembly period mortality for sheep assembled in Adelaide. Sheep received, mortality count and mortality percentage are shown by assembly depot and voyage. These numbers do not include sheep that were dead on arrival at the assembly depot.

Assembly Depot	Voyage	Received	Mortality Count	Mortality %
3	**5**	36,630	17	0.05%
	9	12,634	2	0.02%
	11	38,398	40	0.10%
	12	37,143	35	0.09%
	15	14,862	12	0.08%
	18	14,480	25	0.17%
	21	40,701	15	0.04%
	22	41,252	65	0.16%
	24	45,202	18	0.04%
	Total	**281,302**	**229**	**0.08%**
4	**5**	38,076	2	0.01%
	9	25,750	4	0.02%
	11	38,397	8	0.02%
	12	34,320	7	0.02%
	15	25,266	25	0.10%
	17	28,220	26	0.09%
	21	28,466	9	0.03%
	22	27,824	21	0.08%
	24	30,899	19	0.06%
	Total	**277,218**	**121**	**0.04%**

Table 6-2 Assembly period mortality for sheep assembled in Portland. Sheep received, mortality count and mortality percentage are shown by assembly depot and voyage. These numbers do not include sheep that were dead on arrival at the assembly depot.

Assembly Depot	Voyage	Received	Mortality Count	Mortality %
1	**1**	20,574	15	0.07%
	3	28,946	1	0.003%
	4	27,641	10	0.04%
	6	15,694	4	0.03%
	9	17,440	9	0.05%
	10	12,000	8	0.07%
	19	26,259	50	0.19%
	23	28,685	8	0.03%
	26	20,435	5	0.02%
	27	34,054	16	0.05%
	28	21,449	11	0.05%
	29	30,300	8	0.03%
	30	30,300	8	0.03%
	33	36,432	8	0.02%
	34	27,811	28	0.10%
	Total	**378,020**	**189**	**0.05%**
2	**1**	21,939	5	0.02%
	2	24,823	14	0.06%
	3	30,996	5	0.02%
	4	26,959	3	0.01%
	6	16,706	1	0.01%
	7	5,218	1	0.02%
	9	16,965	1	0.01%
	10	22,029	2	0.01%
	16	18,459	4	0.02%
	21	33,447	10	0.03%
	23	28,221	21	0.07%
	26	40,072	8	0.02%
	27	26,009	11	0.04%
	28	25,938	12	0.05%
	29	21,827	13	0.06%
	30	27,901	6	0.02%
	31	40,807	12	0.03%
	33	9,704	4	0.04%
	35	27,948	17	0.06%
	Total	**465,560**	**150**	**0.03%**

Mortality tends to be highest towards the end of the assembly period. Fig 6.1 shows assembly period mortality by day for all voyages listed in tables 6.1 and 6.2. For these assemblies sheep were received at each assembly depot over a 24 hour period so the number of sheep at risk is similar for each day.

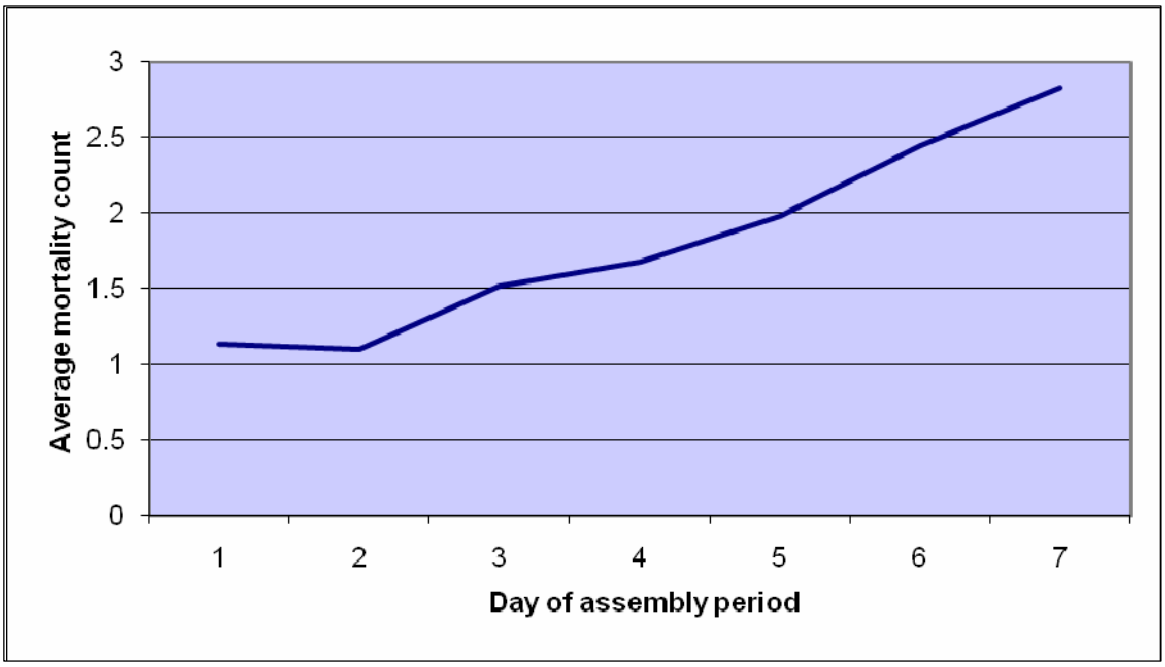


Figure 6-1 Average mortality count by day of assembly period for all voyages excluding the day of receival

6.2.2 Causes of mortality

Table 6.3 and figure 6.2 shows diagnosis by day for the 3 assembly periods investigated. The three most common causes of mortality were trauma, urinary disease and enteritis. It is apparent that trauma is the dominant cause of death at receival while salmonellosis emerges from the middle to the end of the assembly period.

Table 6-3 Receival (dead or euthanized on arrival) and assembly period causes of mortality. Count of mortality is presented by voyage and day since the start of assembly

		Cumulative	Diagnosis			
Voyage	Day	Total	Trauma	Urinary	Enteritis	Other
1	**Receival**	14	**11**	**1**	0	**2**
	1	16	**1**	0	0	**1**
	2	19	0	**1**	0	**2**
	3	23	0	**2**	**1**	**1**
	4	24	0	0	**1**	0
	5	26	0	0	0	**2**
	6	29	0	0	**3**	0
	7	34	0	0	**3**	**2**
5	**Receival**	3	**1**	0	**1**	**1**
	1	5	0	0	0	**2**
	2	7	0	0	0	**2**
	3	20	0	0	**6**	**7**
	4	20	0	0	0	0
	5	22	0	0	**1**	**1**
	6	22	0	0	0	0
	7	22	0	0	0	0
26	**Receival**	16	**9**	**3**	**3**	**1**
	1	18	0	0	**1**	**1**
	2	18	0	0	0	0
	3	22	0	0	**2**	**2**
	4	25	0	**1**	**1**	**1**
	5	27	0	**1**	0	**1**
	6	27	0	0	0	0
	7	29	0	**1**	**1**	0

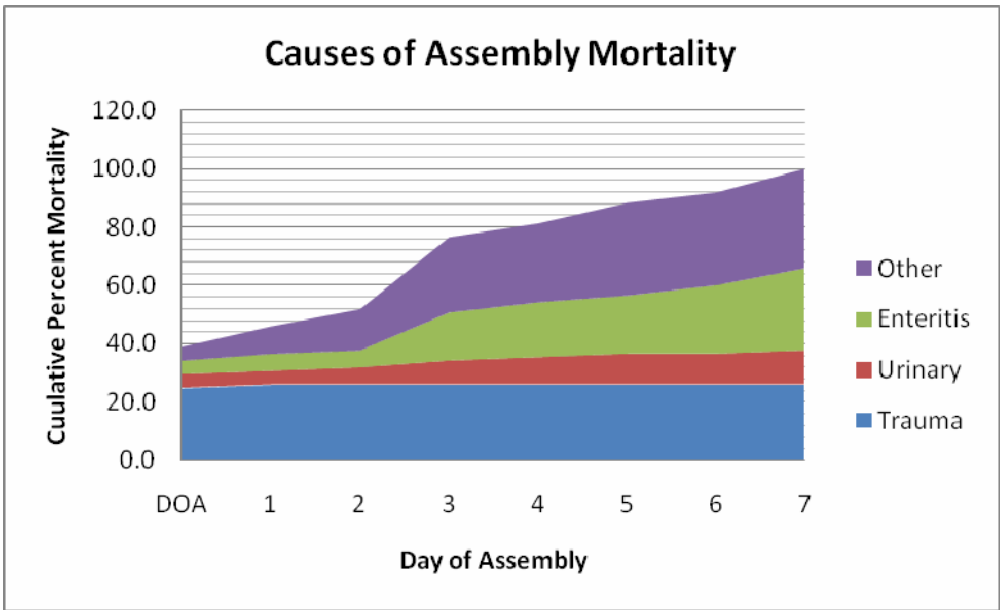


Figure 6-2 **Causes of mortality expressed as a cumulative percentage of all assembly mortality observed during assemblies for voyages 1, 5, and 26**

6.2.3 Salmonella serotypes

Tissue swabs for salmonella culture were collected from post-mortems with gross pathology indicative of enteritis. Samples collected for salmonella culture are summarised in table 6.4 and culture results are outlined in table 6.5. Note the number of samples does not correspond to the number of sheep that died because multiple samples were collected from some animals to investigate the invasiveness (liver, spleen) of the salmonella isolates.

Table 6-4 Assembly period postmortem salmonella culture samples collected and % positive

Voyage	Samples collected	Positives	% Positive
1	21	20	95.2%
5	11	10	90.9%
26	4	1	25.0%

Tabe 6-5 Assembly period postmortem salmonella culture results by voyage showing serotypes isolated and frequency of isolation.

Voyage	Salmonella Serotype	Phage Type	Number Isolated
1	*Salmonella bovismorbificans*	13	5
	Salmonella bovismorbificans	24	5
	Salmonella typhimurium	60	4
	Salmonella typhimurium	RDNC	3
	Salmonella typhimurium	61	2
	Salmonella typhimurium	204	1
5	*Salmonella bovismorbificans*	24	7
	Salmonella typhimurium	135	2
	Salmonella bovismorbificans	32	1
26	*Salmonella typhimurium*	RDNC	1

6.3 Assembly depot salmonellosis

During the assembly period for voyage 22 at assembly depot 6 an outbreak of salmonellosis occurred. In this case there were a large number of carryover sheep in the assembly depot that were held back from the previous voyage, in part due to problems with salmonellosis during the previous assembly period. Due to the shortage of sheep, sheep had been stockpiled over several weeks so the sheep had been in the assembly depot between 8 and 21 days. At receival for voyage 22, clinical disease was observed in carry over lines that had been in the assembly depot 7 – 14 days. At the time of load out the most recently received lines were affected and were subsequently excluded from the shipment.

During the week prior to vessel loading over 300 sheep died in the assembly depot. There were approximately 55,000 sheep in the assembly depot at the time of the outbreak, giving a mortality rate for the week of 0.55% or 55 deaths per 10,000 sheep. Of the 55,000 sheep, approximately 31,800 were loaded onto the vessel. Groups that were showing signs of disease at loading were not loaded onto the vessel. The subsequent mortality rate for the sheep loaded on the ship was low indicating that the drafting process was effective at removing compromised sheep.

Clinical manifestations of disease in the assembly depot included diarrhoea, anorexia, and a hunched stance consistent with abdominal pain. Post-mortem examinations were conducted each day on a sample of sheep and gross pathological findings included diffuse inflammation of the intestinal tract, enlargement of mesenteric lymph nodes, and diarrhoea. A total of 52 tissue swabs were collected from 33 sheep for salmonella culture. Thirty-six samples (69%) from 27 sheep (82%) were positive. The Salmonella serotypes isolated are detailed in table 6.6.

Table 6-6 Salmonella serotypes isolated from assembly period postmortem samples collected during voyage 22.

Salmonella Serotype	Phage Type	Number Isolated
Salmonella bovismorbificans	24	15
Salmonella typhimurium	135	12
Salmonella typhimurium	23	2
Salmonella infantis	-	1
salmonella subsp1 ser 4,5:-:1,2	-	1
Salmonella typhimurium	9	1
Salmonella typhimurium	12	1
Salmonella typhimurium	44	1
Salmonella typhimurium	193	1
Salmonella typhimurium	12a	1

The most common post-mortem isolates were *Salmonella bovismorbificans* 24 and *Salmonella typhimurium* 135. As shown in section 7.2.4, these serotypes were also amongst the most frequently isolated salmonella serotypes from environmental samples taken from all of the assembly depots and from faeces of sheep during the assembly period and from post mortem isolates collected on ships.

PROGRESSION OF CLINICAL SALMONELLOSIS

The length of the assembly period is important as it impacts on the presentation of clinical salmonellosis. When animals are experimentally challenged with salmonella they develop a fever, go off their feed, and develop diarrhoea in 36 – 48 hours. The interval following challenge to the onset of clinical signs is influenced by the salmonella serotype and challenge dose. Generally the larger the challenge dose the shorter the interval. Clinical signs peak between 3 – 7 days following challenge and it is uncommon for animals to die after day 14 post challenge. With a 3 day assembly period, the amount of disease observed at the assembly depot will be relatively minimal if sheep are exposed to salmonella organisms for the first time at the assembly depot. In these instances, the majority of the disease will be observed during the first 7 – 10 days of the voyage. However, in the live export trade, exposure to salmonella will occur over a more prolonged period of time so the onset and duration of disease in the population will be more prolonged.

7 Investigation of Inappetence, Salmonellosis and Inanition

7.1 Materials and methods

7.1.1 General

The relationships between inappetence and salmonellosis incidence in the pre-export assembly depot were primarily investigated during two eastern assembly periods. The Portland investigation was carried out during the assembly period for voyage 1 in September of 2005 and the Adelaide investigation was carried out during the assembly period for voyage 9 in August 2006. For both assembly periods, research lines were included in the consignment.

The investigations included postmortem examinations and collection of samples for salmonella culture and blood biochemical testing. Salmonella culture was performed on post-mortem and faecal

samples as well as samples collected from the environment and feed troughs. Additional environmental cultures were taken at other time points from assembly depots in Adelaide and Fremantle. A summary of blood samples collected for biochemical testing is shown in table 7.1. A summary of all salmonella culture samples collected during assembly period and voyage investigations for the LIVE.123 project is given in table 7.2 (NV = No voyage).

For the West Australian assembly depots, more limited investigations were carried out with environmental sampling for salmonella culture being performed twice.

Table 7-1 Number of blood samples collected from sheep for biochemical analysis during the assembly period for voyages 1 and 9. Year, month, port and assembly depot are also shown.

Year	Month	Port	Voyage Code	Assembly Depot	Samples Collected
2005	**September**	**Portland**	1	1	150
				2	299
2006	**August**	**Adelaide**	9	3	231
				Total	**680**

Table 7-2 **Summary of all environmental, faecal and postmortem samples collected for salmonella culture during assembly depot and voyage investigations for the LIVE.123 project. Year, month, port, voyage code and assembly depot are also shown**

					Sample Type				
Year	Month	Port	Voyage Code	Assembly Depot	Environmental	Faecal	Assembly Postmortem	Voyage Postmortem	Monthly Total
2005	September	Portland	1	1 2	214 188	710 1335	9 12	135	2603
	December	Portland	26	1			6		6
2006	April	Portland Adelaide	4 5	1, 2 3, 4			 11	84	 95
	August	Adelaide	9	3 4	246 47	1255		60	1608
2007	May	Adelaide	15	3, 4	60				60
	June	Adelaide Fremantle	17	3,4 5	45			144	189
	September	Adelaide Fremantle	22	3,4 6	60 47		 52	205	 364
	October	Adelaide	24	3,4	60				60
	November	Adelaide	NV	3,4	90				90
	December	Adelaide Fremantle	37 25	3,4 6	60 20				80
				Total	**1137**	**3300**	**90**	**628**	**5155**

7.1.2 Salmonella culture sampling strategy

Faecal salmonella cultures were carried out to determine the prevalence of faecal salmonella shedding in the assembly depot. Faecal sampling was carried out on a subset of sheep towards the end of the assembly period (1-2 days before load out). Faecal samples were collected randomly from all research lines and a randomly selected sample of sheep from other lines. Faecal samples were collected directly from the rectum and pooled into groups of five for salmonella culture. The expected prevalence of faecal salmonella shedding was used to determine the sampling strategy for faecal salmonella culture. It was determined that culturing 250 faecal samples from each group would allow precise estimation of prevalence of faecal salmonella shedding and detection of significant differences in prevalence between groups. These 250 samples were combined into 50 pools of five for culture. Pooled or group testing is a testing strategy where samples from a number of individuals are aggregated into a single sample (or pool), which is then tested for the disease or agent of interest. Pooled testing offers a cost-effective alternative to testing samples from individual animals, particularly when the prevalence of the outcome of interest is likely to be relatively low. Pooled testing does allow estimation of individual-animal prevalence with comparable precision compared to individual animal testing.

Environmental salmonella culture was carried out to assess the level of environmental contamination with salmonella organisms in the assembly depot and subsequently sheep exposure to salmonella during the pre-assembly period. Environmental samples were collected from a selection of paddocks that contained sheep at each assembly depot. Paddocks were sampled twice, at the beginning of the assembly period and at the end of the assembly period so that early and late samples could be compared. A total of 10 samples were collected from each paddock selected for sampling. Samples collected from each paddock included; one sample of hay from the ground, two samples of pelleted feed and seven ground samples. Ground samples were collected walking seven separate diagonal lines across the paddock; one sample was collected for each diagonal by stopping every 10-15 paces and collecting grass or dirt.

Paddock sampling was found to be labour intensive and traceability to an outcome on ship limited. However, salmonella exposure is required for salmonellosis to occur so an alternate sampling strategy was investigated to provide a measure of assembly depot salmonella exposure over a greater number of voyages. Brief periods of salmonella exposure are sufficient to establish an infection in compromised hosts. All sheep enter and leave assembly depots via the loading ramps and yards. Sampling of these facilities was instigated to provide a measure of salmonella exposure on arrival, salmonella shedding on discharge and persistence of salmonella organisms. This sampling strategy also provided an opportunity to determine which salmonella serovars are prevalent in the assembly depot for comparison to salmonella isolates collected from post mortems conducted on ship. Fifteen samples were collected from the yards of assembly depots 3 and 4 before receival and after load out for all voyages (Voyages 15, 16, 22, and 24) from May to December of 2007. Samples were also collected at roughly fortnightly intervals when no sheep were assembled. The yard samples were collected from the receival/loadout yards. Each yard was divided into an imaginary grid and 18 plus handfuls of yard dirt/faeces collected systematically walking the grid and mixed into a single sample. This process was repeated fifteen times for each time point and for each of the assembly depots. The timing of the sample collection and results of the cultures are presented in Table 7.7

7.1.3 Salmonella culture technique

Salmonella culture was carried out according to laboratory protocols at Elizabeth Macarthur Agricultural Institute. All samples were enriched prior to primary culture. Salmonella were isolated and identified using salmonella specific media, a commercial latex agglutination test and biochemical tests. Salmonella positive samples were sent to the salmonella reference laboratory (Institute of Medical and Veterinary Science, Adelaide) for confirmation of salmonella identity and determination of salmonella serotype.

7.1.4 Salmonella virulence testing

Virulence refers to the relative ability of an infectious agent to cause disease. Virulence testing was carried out to measure the ability of the salmonella serotypes isolated to cause disease. The decision to perform virulence testing was largely opportunistic. A university research group made a request for salmonella isolates for an unrelated salmonella virulence investigation research project. In return for providing a number of salmonella isolates, the results of the virulence assays were made available to the project. This opportunity arose early in 2006, as such virulence testing was only performed on a random selection of isolates from voyage 1 investigations (assembly depot and voyage). The virulence assay compared the virulence of the salmonella isolates to a reference strain of *Salmonella typhimurium* known as universal killer 1 (UK1). Virulence genes were also identified by polymerase chain reaction (PCR).

7.1.5 Antimicrobial susceptibility testing

A subset of faecal, environmental and post-mortem samples were tested to determine the susceptibility of live export salmonella isolates to a variety of antimicrobial agents commonly used to treat salmonella infections. Antimicrobial testing was conducted for two reasons, firstly to investigate the prevalence of antimicrobial resistance with a view to therapeutic management and secondly to identify isolates that could be utilised in the proliferation experiment (described below). Antimicrobial sensitivity testing was carried out by the disk diffusion method.

7.1.6 Salmonella proliferation experiment

To further understand environmental salmonella contamination, a laboratory study was undertaken to quantify survival and proliferation of salmonella and determine if moisture and temperature influence the number of salmonella present in the faecal pack that builds up in sheep yards and on ship. This was achieved through quantitative salmonella culture in which salmonella numbers are measured in colony forming units (CFU) per gram of faecal pack.

Faeces used in this experiment were collected from sheep consuming export pellets and were shown to be free of salmonella organisms on multiple enrichment cultures. Two salmonella isolates with known resistance to antimicrobials were chosen. Antimicrobials the salmonella were resistant to were incorporated into culture media to create a selective media for enumeration experiments. Two salmonella serovars were evaluated, *Salmonella bovismorbificans* obtained from a sheep at post mortem and *Salmonella typhimurium* obtained from the environment of one of the assembly depots.

The faeces were dried and split into four 7 gram samples to which were added sterile saline to give 4 samples of different moisture contents (0%, 15%, 30% and 60%). Each of the 4 samples were duplicated and inoculated with either S. bovismorbificans or S. typhimurium. For each sample, quantitative culture was used to determine the number of salmonella per gram of faeces at the time of inoculation and after 8 hours, 24 hours, 72 hours, 7 days and 21 days at room temperature and 37^{o}C.

7.1.7 Serum biochemistry

Blood biochemistry testing was aimed at assessing energy balance through evaluation of serum non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BOHB). Tissues can use carbohydrates, amino acids (proteins) and fats as energy sources. In ruminants, carbohydrates are the main energy source. When insufficient energy is consumed to meet the animal's needs (inappetence or feed restriction) negative energy balance results. Some degree of negative energy balance is inherent in the management associated with the transport of sheep from property of origin to the assembly depot. Management procedures that may interrupt feed intake include mustering, yarding, curfews and road transport. Negative energy balance can lead to ketosis and if prolonged for weeks, to inanition. Inanition is defined as an exhausted state of prolonged under nutrition or starvation (Blood and Studdert 1999). In negative energy balance, body protein (muscle) and fat are metabolised to produce energy, carbohydrates can be synthesised from protein but not from fat. Synthesis of carbohydrate from body proteins is limited to prevent depletion of body proteins.

Body fat contains triglycerides which consist of 3 long-chain fatty acids and a glycerol back bone. In negative energy balance an enzyme called lipase is activated and triglycerides are broken down (lipolysis) into NEFA and glycerol (Herdt 2000). The glycerol component can be converted by the liver to glucose (Beitz 1993). NEFA and glycerol can also be re-esterified (lipogenesis) to triglycerides.

In peripheral (non-hepatic) tissues NEFA prevents uptake and utilisation of glucose and can be used as an alternative energy source. If the triglycerides remain in the liver, fatty liver and liver dysfunction can result (Herdt 2000). In the liver, negative energy balance allows activation of enzymes that facilitate the transport of NEFA into hepatic mitochondria where they are converted to ketones, including BOHB. BOHB can be used as an energy source. When the concentration of ketones in the blood is elevated (a state called ketosis), altered mental state can lead to further depression of appetite in an already inappetent animal (Radostits, Gay et al. 2000). This process is portrayed in figure 7.1.

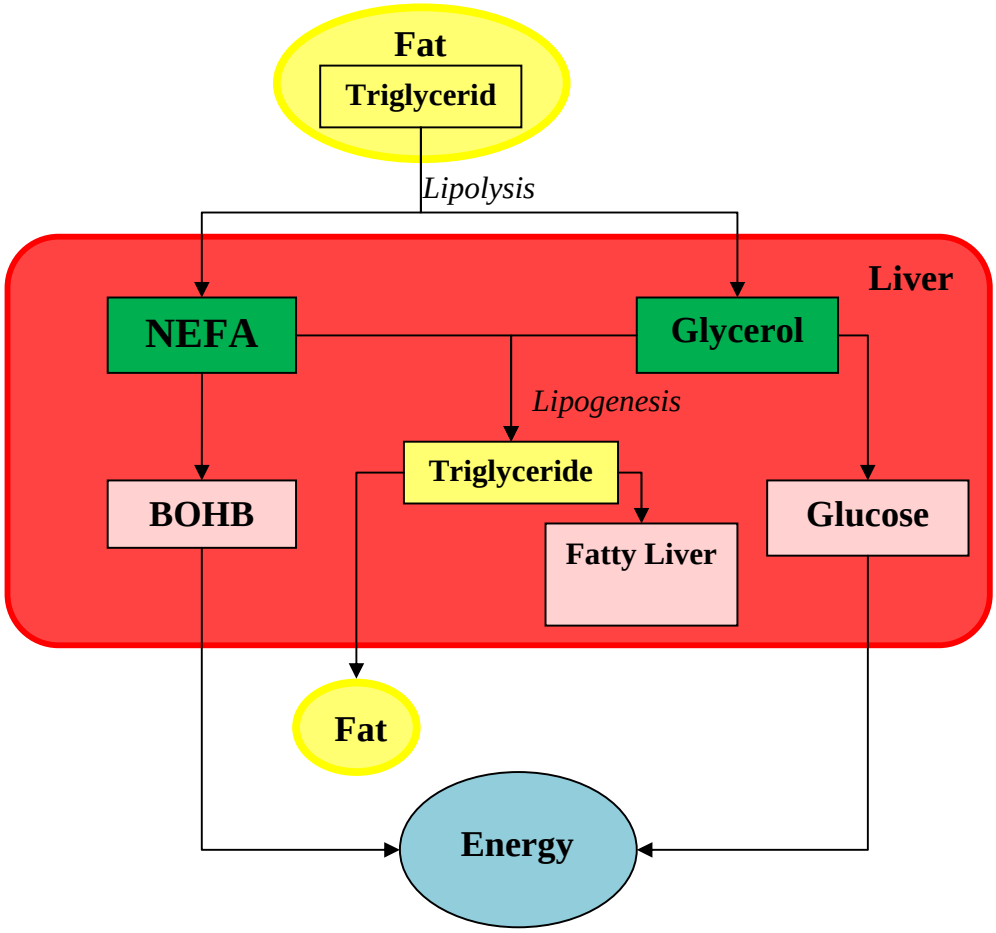


Figure 7-1 Schematic representation of energy metabolism in ruminants

Not all animals in negative energy balance will become ketotic. Prolonged or absolute lack of dietary energy is required to produce clinical ketosis in sheep. Ketosis in sheep is usually limited to ewes in late gestation (pregnancy toxaemia) where the overwhelming energy demands of multiple foetuses cannot be met by dietary intake. There is strong evidence to suggest that non-pregnant sheep cope well with prolonged energy deprivation. Moloney and Moore (1994) found that prolonged (8 weeks) restriction of energy to 56 kg crossbred Suffolk wether lambs (feeding 25% of that required for maintenance) did not result in sustained elevation in blood BOHB or clinical ketosis (Moloney and Moore 1994). In the same study prolonged feed restriction did cause a consistent increase in the concentration of NEFA's from 0.1 mmol/L to between 0.4 and 0.7 mmol/L. This increase was less than the 1 - 2 mmol/L previously observed in sheep with "inanition" in the live sheep trade.(Richards, Hyder et al. 1991) It has been shown that complete fasting of sheep for 3 days will cause BOHB to increase to greater than 0.7mmol/L (Warriss, Bevis et al. 1989), however this is still well below the levels of BOHB (>3mmol/L) observed in clinically ketotic sheep (Radostits, Gay et al. 2000). It appears that most sheep which remain persistently inappetent will go on to die from inanition (exhaustion of body stores and circulatory failure) without developing clinical ketosis.

As described above serum NEFA will be elevated in sheep that are in negative energy balance and are mobilising fat reserves. BOHB is typically elevated in more prolonged or severe negative energy balance. NEFA and BOHB analyses were performed by the University Veterinary Centre Camden

Pathology Laboratory. In addition to this, inflammatory response was assessed by evaluation of serum haptoglobin which was tested using a commercial ELISA test kit (Tri-Delta).

7.2 Salmonella culture results

7.2.1 Faecal culture

The results of the pooled culture were used to estimate true prevalence of salmonella faecal shedding and are presented in table 7.3. The prevalence of faecal shedding was estimated from the pooled culture results using the online pooled prevalence calculator at (http://www.ausvet.com.au/content.php?page=epitools).

Table 7-3 Faecal salmonella culture results showing pools tested, number positive and estimated prevalence of faecal shedding with 95% confidence intervals by group

Port	Group	Pools Tested	Positive Pools	Estimated Prevalence of Faecal Shedding	95% CI Low	95% CI High
Portland	Total	409	42	0.03	0.020	0.041
	Research Lines	102	3	<0.01	0.000	0.018
	Other Lines	307	39	0.04	0.026	0.053
Adelaide	Total	251	29	0.03	0.021	0.049
	Research Lines	150	6	0.01	0.002	0.021
	Other Lines	101	23	0.07	0.042	0.109

The proportion of positive samples for each group was compared to determine if there are any differences in salmonella shedding between groups. Table 7.4 summarises the groups sampled for faecal salmonella culture and the proportion (p) of samples that were positive. The class of sheep (A, B, or C) refers to the average estimated weight and condition of the animals with A-class wethers being the heaviest and C-class being the lightest. The pastoral, distance and lamb classes listed in this table are all research lines. Groups listed as non-pastoral represent sheep included in the same paddock as pastoral sheep for comparison. The 95% confidence intervals (CI) were estimated using the method described by Wilson (Brown, Cai et al. 2001).

Table 7-4 Faecal salmonella culture results by group showing class of sheep, number of pools tested (n) and proportion positive (p) with 95% CI

Port	Assembly Depot	Group	Class	n	Positives	p	Wilson 95% CI Lower	Wilson 95% CI Upper
Portland	**1**	**1**	C Wethers	46	0	0.00	0.000	0.077
		2	A Wethers	46	0	0.00	0.000	0.077
		3	Young B Wethers	50	0	0.00	0.000	0.071
	2	**4**	Young Non-Pastoral	31	31	1.00	0.890	1.000
		5	Old Pastoral	49	1	0.02	0.004	0.107
		6	Old Non-Pastoral	42	0	0.00	0.000	0.084
		7	Young Pastoral	53	2	0.04	0.010	0.128
		8	C Wethers	46	7	0.15	0.076	0.282
		9	B Wethers	46	1	0.02	0.004	0.113
Adelaide	**3**	**10**	Pastoral Wethers	50	1	0.02	0.004	0.105
		11	Lambs	50	2	0.04	0.011	0.135
		12	A Wethers	50	21	0.42	0.294	0.558
		13	Distance Wethers	50	3	0.06	0.021	0.162
		14	A Young Wethers	51	2	0.04	0.011	0.132

Figure 7-2 Faecal salmonella culture proportion positive by group with 95% CI

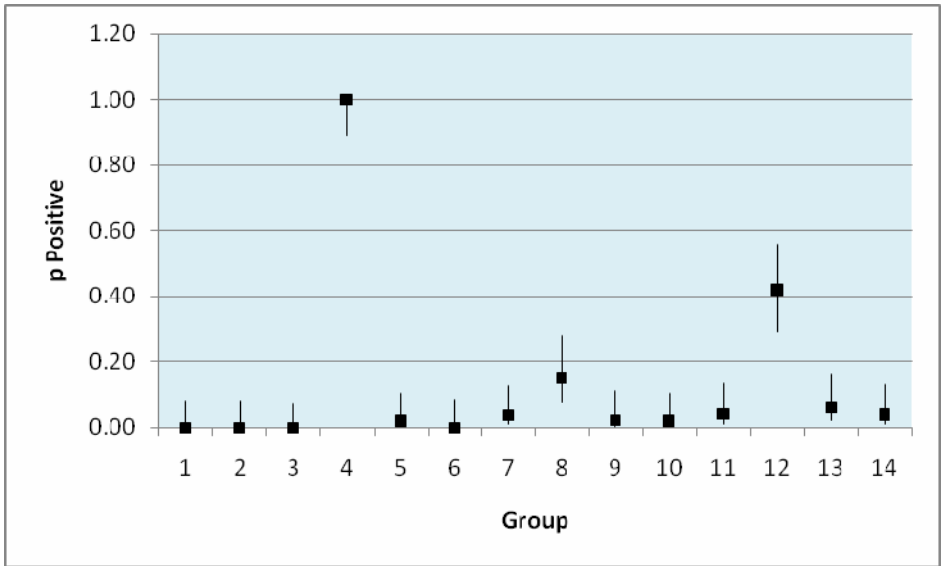


Figure 7.2 is a graphical representation of the data presented in table 7.4. The squares represent the proportion positive and the lines represent the confidence interval.

Figure 7.2 shows that groups 4 and 12 have a significantly higher proportion of positive culture results than all other groups. Results of salmonella serotyping and number of each serotype isolated from faecal samples are shown in table 7.5.

Table 7-5 Salmonella serotypes isolated from faecal samples by port with number isolated

Port	Salmonella Serotype	Phage Type	Number Isolated
Portland	*Salmonella bovismorbificans*	24	34
	Salmonella bovismorbificans	9	5
	Salmonella typhimurium	61	1
	Salmonella bredeney	-	1
	Salmonella Singapore	-	1
	Salmonella Subsp 3b ser 61:1, v:z35	-	1
Adelaide	*Salmonella bovismorbificans*	24	15
	Salmonella bovismorbificans	32	2
	Salmonella bovismorbificans	Untypable	1
	Salmonella typhimurium	135	7
	Salmonella typhimurium	60	1
	Salmonella typhimurium	9	1
	Salmonella anatum	-	1
	Salmonella saintpaul	-	1

7.2.2 Paddock culture

A summary of paddock samples collected and culture results are shown in table 7.6 below.

Table 7-6 Paddock salmonella culture results by voyage, assembly depot and timing of sample collection, showing number collected (n) and proportion positive (p) with 95% CI

		Assembly					Wilson 95% CI	
Port	**Voyage**	**Depot**	**Timing**	**N**	**Positives**	**p**	**Lower**	**Upper**
Portland	**1**	**1**	Early	145	5	0.03	0.015	0.078
			Late	69	5	0.07	0.031	0.159
		2	Early	118	14	0.12	0.072	0.189
			Late	70	17	0.24	0.158	0.355
Adelaide	**9**	**3**	Early	130	4	0.03	0.012	0.076
			Late	116	16	0.14	0.087	0.212
		4	Early	30	1	0.03	0.006	0.167
			Late	17	5	0.29	0.133	0.531
		All	Early	423	24	0.06	0.038	0.083
			Late	272	43	0.16	0.120	0.206
			Total	695	67	0.10	0.077	0.121

Figure 7.3 Proportion of salmonella positive samples for the early and late round of sampling in each of the four assembly depots. In both investigations the overall proportion of positive salmonella cultures increased during the assembly period indicating that salmonella contamination and sheep exposure increases during the assembly period.

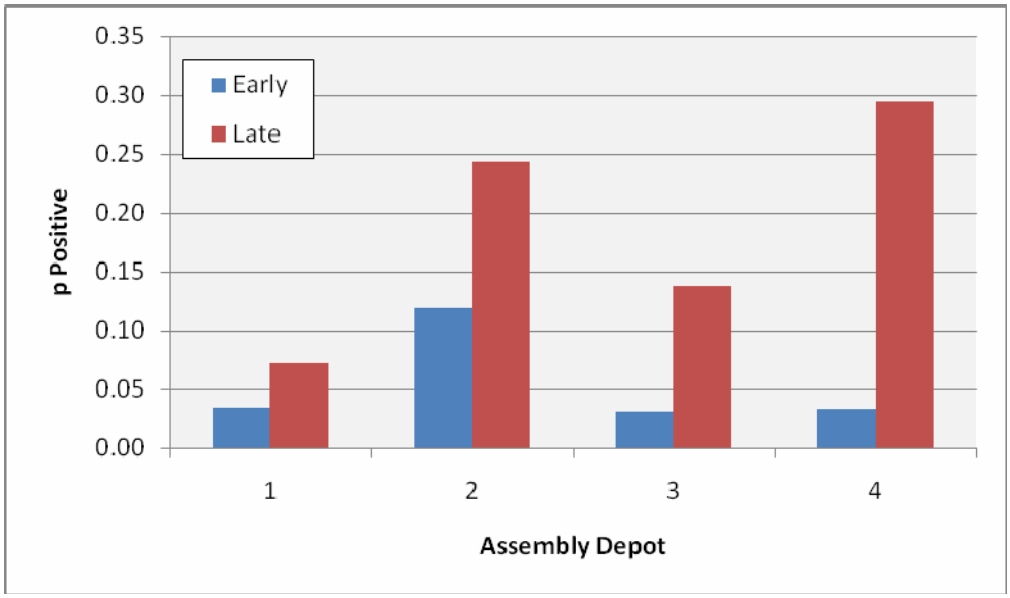


Figure 7-3 Paddock salmonella culture results, proportion positive by assembly depot and time of sampling Image quality not the best

7.2.3 Yards culture

A summary of yard samples collected and salmonella culture results are shown in table 7.7. In this table, “no ship” refers to a sampling that occurred at a time point when no sheep were assembled. More salmonella were isolated from both assembly depots during the time of highest throughput of sheep, May to October. During this period, the assembly depots received regular rainfall and had mild ambient temperatures. The association between high sheep throughput and increased risk of salmonellosis is logical during weather conditions that are conducive to the survival / proliferation of the organism given that contamination increases over the duration of the assembly period. The number of positive samples gradually dropped off as fewer sheep passed through the yards (samples taken when there was no shipment) and temperature increased. During summer (December) salmonella contamination was low (or undetectable) when the yards were sampled prior to receivals and increased during the assembly period.

Table 7-7 Yard salmonella culture results by voyage, assembly depot and timing of sample collection showing number collected (n) and proportion positive (p) with 95% CI

			Assembly					Wilson 95% CI	
Year	Month	Voyage	Depot	Timing	n	Positives	P	Lower	Upper
2006	**August**	**9**	**3**	Receival	15	1	0.07	0.012	0.298
			3	Load Out	15	1	0.07	0.012	0.298
			4	Receival	14	1	0.07	0.013	0.315
			4	Load Out	17	5	0.29	0.133	0.531
2007	**May**	**15**	**3**	Receival	15	6	0.40	0.198	0.643
			3	Load Out	15	8	0.53	0.301	0.752
			4	Receival	15	7	0.47	0.248	0.699
			4	Load Out	15	14	0.93	0.702	0.988
	June	**17**	**3**	Receival	15	9	0.60	0.357	0.802
			3	Load Out	15	2	0.13	0.037	0.379
			4	Receival	15	13	0.87	0.621	0.963
	September	**22**	**3**	Receival	15	6	0.40	0.198	0.643
			3	Load Out	15	6	0.40	0.198	0.643
			4	Receival	15	9	0.60	0.357	0.802
			4	Load Out	15	12	0.80	0.548	0.930
	October	**24**	**3**	Receival	15	10	0.67	0.417	0.848
			3	Load Out	15	14	0.93	0.702	0.988
			4	Receival	15	12	0.80	0.548	0.930
			4	Load Out	15	12	0.80	0.548	0.930
	November		**3**	No Ship	15	8	0.53	0.301	0.752
			4	No Ship	15	6	0.40	0.198	0.643
			3	No Ship	15	6	0.40	0.198	0.643
			4	No Ship	15	7	0.47	0.248	0.699
			3	No Ship	15	8	0.53	0.301	0.752
			4	No Ship	15	4	0.27	0.109	0.520
	December	**37**	**3**	Receival	15	2	0.13	0.037	0.379
			3	Load Out	15	8	0.53	0.301	0.752
			4	Receival	15	0	0.00	0.000	0.204
			4	Load Out	15	8	0.53	0.301	0.752

Results of salmonella serotyping and number of each serotype isolated from environmental samples (both paddocks and yards) are shown in table 7.8.

Table 7-8 Salmonella serotypes isolated from environmental samples by port and assembly depot with number isolated

Port	Assembly Depot	Salmonella Serotype	Phage Type	# Isolated
Portland	1	*Salmonella kottbus*	-	6
		Salmonella typhimurium	9	5
		Salmonella subsp 3b ser rough:1, v:z35	-	1
Portland	2	*Salmonella Infantis*	-	9
		Salmonella bovismorbificans	24	7
		Salmonella bovismorbificans	13	5
		Salmonella bredeney	-	3
		Salmonella typhimurium	9	3
		Salmonella bovismorbificans	32	1
		Salmonella subsp 3b ser rough:1, v:z35	-	1
		Salmonella tennessee	-	1
		Salmonella typhimurium	61	1
Adelaide	3	*Salmonella bovismorbificans*	24	25
		Salmonella newport	-	19
		Salmonella tennessee	-	9
		Salmonella typhimurium	135	16
		Salmonella typhimurium	193	6
		Salmonella typhimurium	12a	9
		Salmonella anatum	-	4
		Salmonella bovismorbificans	32	4
		Salmonella typhimurium	9	7
		Salmonella infantis	-	3
Adelaide	3	*Salmonella typhimurium*	108	3
		Salmonella bovismorbificans	39	1
		Salmonella bovismorbificans	11	1
		Salmonella derby	-	1
		Salmonella havana	-	1
		Salmonella ohio	-	1
		Salmonella subsp1 ser rough:r:1,5	-	1
		Salmonella typhimurium	194	1
		Salmonella typhimurium	RDNC	1
Adelaide	4	*Salmonella bovismorbificans*	24	46
		Salmonella typhimurium	135	26
		Salmonella bovismorbificans	11	7

Port	Assembly Depot	Salmonella Serotype	Phage Type	# Isolated
		Salmonella typhimurium	12a	4
		Salmonella subsp 1 ser 16:l,v:-	-	3
		Salmonella bovismorbificans	40	2
		Salmonella mbandaka	-	2
		salmonella subsp 1 ser rough:r:1,5	-	2
		Salmonella typhimurium	12	2
		Salmonella typhimurium	135a	2
		Salmonella aberdeen	-	1
		Salmonella adelaide	-	1
		Salmonella bovismorbificans	13	1
		Salmonella bovismorbificans	29	1
		Salmonella havana	-	1
		Salmonella Infantis	-	1
		Salmonella newport	-	1
		Salmonella onderstepoort	-	1
		Salmonella oranienburg	-	1
		Salmonella orion	-	1
		Salmonella typhimurium	9	1
		Salmonella typhimurium	60	1
		Salmonella typhimurium	150	1
		Salmonella typhimurium	108	1
Fremantle	6	*Salmonella bovismorbificans*	24	9
		Salmonella typhimurium	135	5
		Salmonella typhimurium	12	3
		Salmonella typhimurium	194	2
Fremantle	6	*Salmonella havana*	-	2
		Salmonella infantis	-	2
		Salmonella typhimurium	135a	2
		Salmonella typhimurium	182	1
		Salmonella typhimurium	12a	1
		Salmonella singapore	-	1
		Salmonella anatum	-	1

7.2.4 Serotypes

Table 7.9 shows the most commonly isolated salmonella serotypes, infrequently isolated serotypes and those not commonly associated with disease in sheep are excluded. Complete lists of isolates from faecal, environmental and tissue samples are shown under the relevant sections.

Table 7-9 The most commonly isolated salmonella serotypes and phage types listed by voyage, port and sample type.

Voyage	Port	Sample Type	Salmonella Serotype	Phage Type
1	**Portland**	Faecal	*Salmonella bovismorbificans*	24
		Environmental	*Salmonella infantis*	-
			Salmonella bovismorbificans	24
		Assembly PM	*Salmonella bovismorbificans*	13
			Salmonella bovismorbificans	24
		Voyage PM	*Salmonella bovismorbificans*	24
			Salmonella typhimurium	135
4	**Portland**	Voyage PM	*Salmonella typhimurium*	135
			Salmonella bovismorbificans	24
5	**Adelaide**	Assembly PM	*Salmonella bovismorbificans*	24
9	**Adelaide**	Faecal	*Salmonella bovismorbificans*	24
		Environmental	*Salmonella bovismorbificans*	24
			Salmonella bovismorbificans	32
			Salmonella typhimurium	135
		Voyage PM	*Salmonella bovismorbificans*	24
			Salmonella typhimurium	9
Various	**Adelaide**	Yards	*Salmonella bovismorbificans*	24
			Salmonella typhimurium	135
			Salmonella newport	-
22	**Fremantle**	Environmental	*Salmonella bovismorbificans*	24
			Salmonella typhimurium	135
		Assembly PM	*Salmonella bovismorbificans*	24
			Salmonella typhimurium	135
		Voyage PM	*Salmonella typhimurium*	135
			Salmonella bovismorbificans	24
			Salmonella anatum	-

Table 7.9 demonstrates that the most common salmonella isolates across all voyages and sample types were *Salmonella bovismorbificans* 24 and *Salmonella typhimurium* 135. This finding is consistent with the results of other investigations in which these serotypes were shown to be pathogenic in sheep (Wray and Davies 2000). Pathogenic serotypes are those which have the ability to cause disease in sheep. Pathogenic serotypes were isolated from the environment at all assembly depots and were also found to be shed in faeces. At least one potentially pathogenic serotype was found in faeces for all groups sampled in Adelaide and 3 of the 9 groups sampled in Portland.

For voyages 1, 9 and 22 salmonella culture information was available for the assembly period and the voyage. In these voyages the same isolates that were present in the assembly depot were found to cause clinical salmonellosis and mortality during the voyage.

7.2.5 Virulence

The results of virulence testing are shown in tables 7.10 and 7.11 below. Isolates obtained from the assembly depot are shown in table 7.10 and isolates obtained from the voyage are shown in table 7.11. The larger the competitive index, the more virulent the salmonella serotype. Logically, virulent isolates were found to possess virulence genes including the salmonella virulence plasmid. None of the 3 isolates obtained from the environment were found to be virulent. However, 1 of the 2 isolates obtained from faeces was found to be virulent and 10 of the 19 isolates obtained from postmortem samples were virulent.

Table 7-10 Results of virulence testing for salmonella isolates obtained during the assembly period for voyage 1 by sample type and assembly depot. For each isolate serotype, virulence, competitive index and presence of virulence plasmid (+/-) are shown.

Sample Type	Assembly Depot	Salmonella serotype	Virulence	Competitive Index	
Faecal	2	*Typhimurium 9*	virulent	0.545	+
	2	*Singapore*	avirulent	<0.0003	-
Environmental	2	*Bovismorbificans 13*	avirulent	<0.0003	+
	2	*Bredeney*	avirulent	<0.0003	-
	1	*Kottbus*	avirulent	<0.0003	-
	2	*Bovismorbificans 32*	avirulent	<0.0003	+
	2	*Infantis*	avirulent	<0.0003	-
Postmortem	1	*Typhimurium 204*	virulent	2.03	+
	2	*Typhimurium 61*	virulent	0.951	+
	1	*Typhimurium 60*	virulent	0.327	+
	2	*Bovismorbificans 13*	avirulent	<0.0003	+

Table 7-11 Results of virulence testing for salmonella isolates obtained during voyage 1 by sample type and port of loading. For each isolate serotype, virulence, competitive index and presence of virulence plasmid (+/-) are shown.

Sample Type	Port of Loading	Salmonella serotype	Virulence	Competitive Index	Virulence Plasmid
Postmortem	**Portland**	*Havana*	avirulent	<0.0003	-
		Havana	avirulent	<0.0003	-
		Bovismorbificans 13	avirulent	<0.0003	+
		Bredeney	avirulent	<0.0003	-
Postmortem	**Fremantle**	*Bovismorbificans 24*	virulent	2.716	+
		Typhimurium 30	virulent	2.32	+
		Bovismorbificans 32	virulent	1.733	+
		Typhimurium 195	virulent	0.726	+
		Typhimurium RDNC	virulent	0.32	+
		Bovismorbificans 24	virulent	0.29	+
		Typhimurium 105 var	virulent	0.219	+
		Havana	avirulent	<0.0003	-
		Bovismorbificans 24	avirulent	<0.0003	+
		Infantis	avirulent	<0.0003	-
		Bovismorbificans 32	avirulent	<0.0003	+
		Infantis	avirulent	<0.0003	+
		Bovismorbificans 13	avirulent	<0.0003	+

7.2.6 Antimicrobial susceptibility

The results of salmonella antimicrobial susceptibility testing are shown in table 7.12. According to the relatively small subset of isolates tested, antimicrobial resistance was not common in salmonella organisms isolated from live export samples with 77% of isolates susceptible to all antibiotics tested. Furthermore, only one isolate was found to have resistance to multiple classes of antibiotic.

Table 7-12 Results of salmonella antimicrobial susceptibility testing

S = susceptible R = resistant AMP = ampicillin SF = sulfamethoxazole W = trimethoprim TE = tetracycline N = neomycin AP = ampicillin EFT = ceftiofur

Sample Type	Salmonella Serotype	Antibiotic						
		AMP	SF	W	TE	N	AP	EFT
Faecal	*Salmonella Typhimurium 9*	S	R	S	S	S	S	S
Post-mortem	*Salmonella Bovismorbificans 24*	S	S	S	S	S	S	S
	Salmonella Bovismorbificans 24	S	S	S	S	S	S	S
	Salmonella Bovismorbificans 24	S	S	S	S	S	S	S
	Salmonella Bovismorbificans 24	S	R	S	S	S	S	S
	Salmonella Bovismorbificans 32	S	S	S	S	S	S	S
	Salmonella Typhimurium 105 var	S	S	S	S	S	S	S
	Salmonella Typhimurium 195	S	S	S	S	S	S	S
	Salmonella Typhimurium 204	S	S	S	S	S	S	S
	Salmonella Typhimurium 30	S	S	S	S	R	S	S
	Salmonella Typhimurium RDNC	S	S	S	S	S	S	S
Environmental	*Salmonella Anatum*	S	S	S	S	S	S	S
	Salmonella Bovismorbificans 13	S	S	S	S	S	S	S
	Salmonella Bovismorbificans 24	S	S	S	S	S	S	S
	Salmonella Bovismorbificans 24	S	S	S	S	S	S	S
	Salmonella Havana	S	S	S	S	S	S	S
	Salmonella Infantis	S	S	S	S	R	S	S
	Salmonella Kottbus	S	S	S	S	S	S	S
	Salmonella Tennessee	S	S	S	S	S	S	S
	Salmonella Typhimurium 193	R	R	S	R	S	S	S
	Salmonella Typhimurium 60	S	S	S	S	S	S	S
	Salmonella Typhimurium 9	S	S	S	S	S	S	S

7.2.7 Salmonella proliferation experiment

Figures 7.5 and 7.6 depict the results of the salmonella proliferation experiment for *Salmonella bovismorbificans* and *Salmonella typhimurium* respectively. The blue dashed line on each graph shows the number of salmonella inoculated. The 30% moisture sample has been omitted from the graphs to reduce the number of overlapping lines.

The significant findings included rapid proliferation of both serotypes following addition of moisture to the samples. The most rapid and largest proliferation was observed in the 60% moisture samples incubated at 37^{o}C. Proliferation was observed in all samples for 1 to 7 days. Moisture appeared to be a bigger driver than temperature as a requirement for proliferation to occur. Proliferation was more rapid at 37^{o}C but persistence was shorter. Persistence was observed through the 21-day study period in the dryer samples.

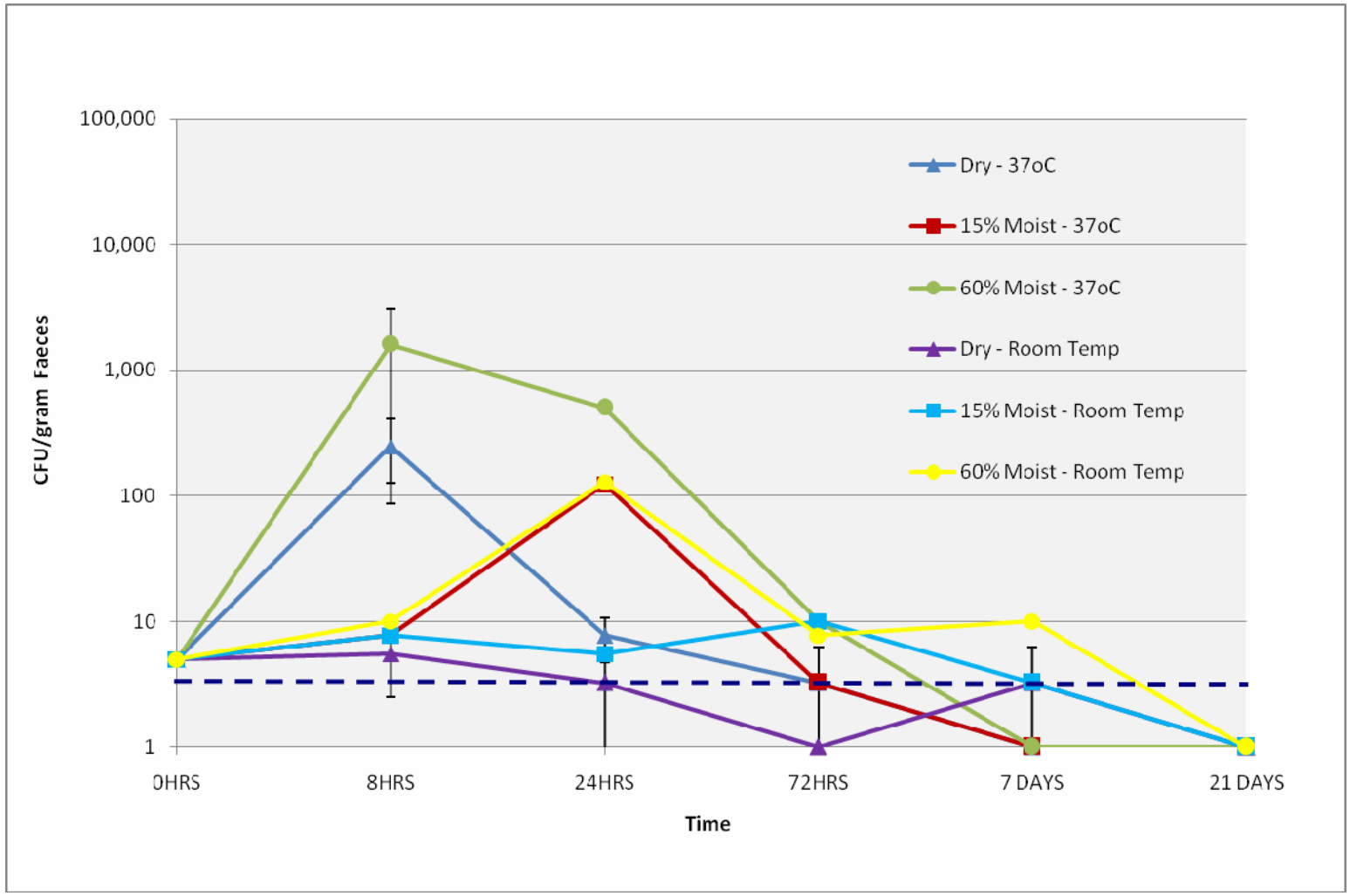


Figure 7-4 Proliferation and persistence of Salmonella bovismorbificans (isolated from a postmortem sample) at various temperatures and moistures

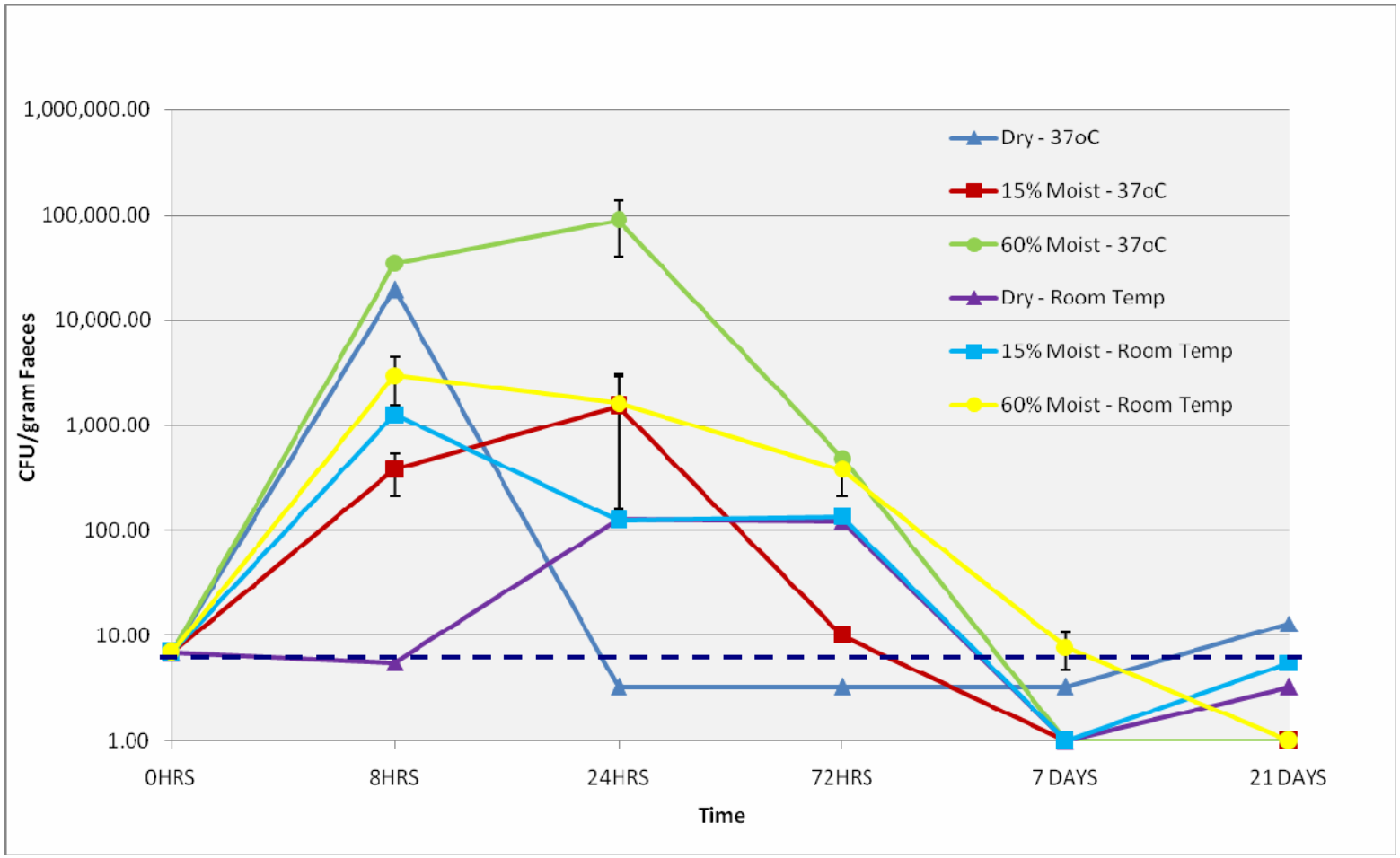


Figure 7-5 Proliferation and persistence of Salmonella typhimurium (isolated from a environment) at various temperatures and moistures

7.3 Serum biochemistry results

7.3.1 Non-esterified fatty acids

A summary of serum samples collected by group and NEFA results is shown in table 7.13. Previous studies conducted in West Australia found that NEFA values for sheep that were observed to be eating ranged from 0.6 to 0.8 mmol/L, while sheep that were inappetent had values ranging from 1.0 to 2.0 mmol/L (Richards, Hyder et al. 1991). These reference ranges have been used in the current study. The pastoral, distance and lamb classes listed in this table are all research lines. Groups listed as non-pastoral represent sheep included in the same paddock as pastoral sheep for comparison.

Although there were sheep in all groups with elevated serum NEFA (greater than 1.0mmol/L), certain groups of sheep were shown to have higher average serum NEFA as well as a higher proportion of individual NEFA result above 1mmol/L. The true mean NEFA for groups 12, 4 and 6 was greater than 1mmol/L. Figure 7.6 compares the proportion of serum NEFA > 1 mmol/L for research lines and other lines.

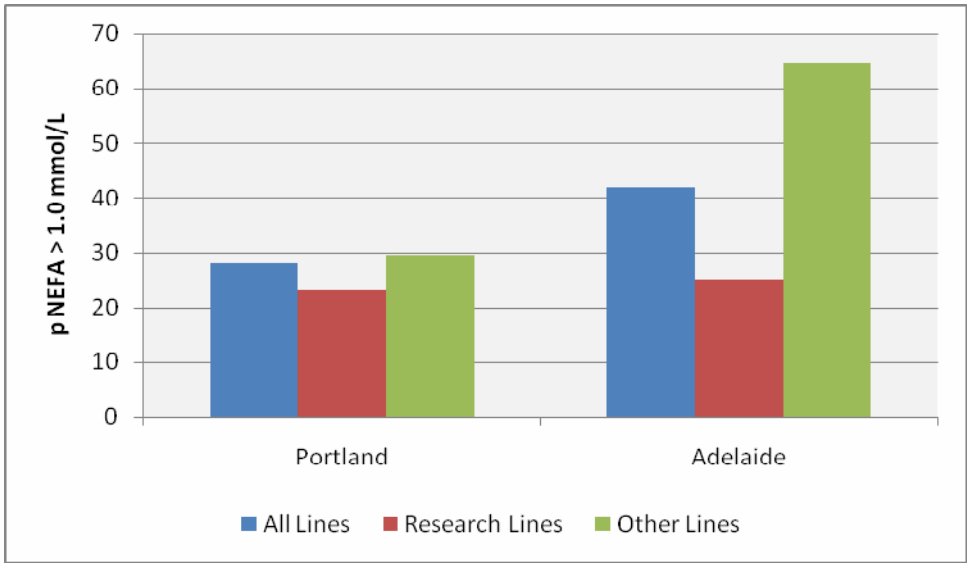


Figure 7-6 Serum NEFA results for Portland and Adelaide showing the proportion of NEFA samples greater than 1.0 mmol/L for all lines, research lines and other lines of sheep image quality

Table 7-13 NEFA results by group showing class, number of samples taken, geometric mean for group and proportion (p) of samples greater than 1.0mmolL with 95% CI

Assembly					Mean#	95% CI		Samples >1mmol/L		95% CI	
Port	Depot	Group	Class	n	mmol/L	Low	High	n	p	Low	High
Portland	**1**	**1**	C Wethers	50	0.56	0.47	0.68	9	0.18	0.098	0.308
		2	A Wethers	50	0.38	0.31	0.46	2	0.04	0.011	0.135
		3	Young B Wethers	50	0.42	0.33	0.53	11	0.22	0.128	0.352
	2	**4**	Young Non-Pastoral	49	1.09	0.91	1.31	35	0.71	0.576	0.821
		5	Old Pastoral	50	0.69	0.59	0.80	15	0.30	0.191	0.438
		6	Old Non-Pastoral	50	0.90	0.77	1.07	25	0.50	0.366	0.634
		7	Young Pastoral	50	0.57	0.50	0.66	8	0.16	0.083	0.285
		8	C Wethers	50	0.34	0.27	0.43	8	0.16	0.083	0.285
		9	B Wethers	48	0.52	0.41	0.66	12	0.25	0.149	0.388
Adelaide	**3**	**10**	Pastoral Wethers	50	0.60	0.49	0.73	14	0.28	0.175	0.417
		11	Lambs	33	0.50	0.40	0.62	8	0.24	0.128	0.410
		12	A Wethers	49	1.32	1.19	1.47	42	0.86	0.733	0.929
		13	Distance Wethers	48	0.65	0.55	0.78	11	0.23	0.133	0.365
		14	A Young Wethers	50	0.87	0.74	1.02	22	0.44	0.312	0.577
			Total	677	0.63	0.59	0.66	222	0.33	0.294	0.364

Geometric mean - a geometric mean is used as NEFA results needed to be transformed to a natural log scale for analysis

Analysis of variance (ANOVA) was used to examine NEFA concentrations between groups of sheep. Results indicated a significant between groups effect ($p<0.001$) and follow-up analyses were then conducted to compare mean values of NEFA (on a natural log transformed scale) between groups with Bonferroni adjustment to correct for multiple comparisons. Model checking included inspection of group specific variance estimates to check for homoscedasticity and inspection of a plot of standardised residuals vs. predicted values to check for normality of residuals and for evidence of any patterns. No problems were detected in these checks.

Table 7.14 shows the p-values for comparisons between groups, red values indicate that a significant difference exists between the two groups. The analysis tells us that the average NEFA for group:

- 12 is significantly higher than all groups except 4 and 6
- 4 is significantly higher than all groups except 5, 6, 12 and 14
- 6 is significantly higher than 1, 2, 3, 8, 9 and 11
- 14 is significantly higher than 2, 3, 8, 9 and 11

The distribution of the NEFA values for all groups are presented in figure 7.7.

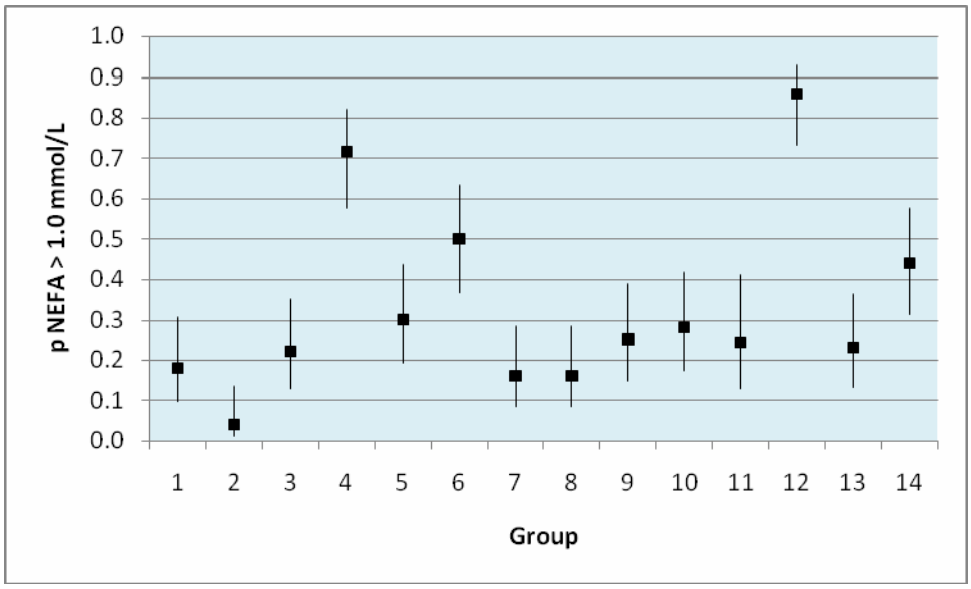


Figure 7-7 Serum NEFA results by Group Proportion of serum NEFA samples greater than 1.0mmol/L with 95% CI

Table 7-14 Of ANOVA for serum NEFA analysis..P-values from Bonferroni adjusted comparisons of log transformed NEFA concentrations for each of 14 different groups, performed as a series of follow-up tests after a one way ANOVA

Group	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.34												
3	1	1											
4	**<0.001**	**<0.001**	**<0.001**										
5	1	**0.001**	**0.02**	0.06									
6	**0.03**	**<0.001**	**<0.001**	1	1								
7	1	0.204	1	**<0.001**	1	0.06							
8	**0.02**	1	1	**<0.001**	**<0.001**	**<0.001**	**0.01**						
9	1	1	1	**<0.001**	1	**0.004**	1	0.17					
10	1	0.07	0.70	**0.001**	1	0.19	1	**0.002**	1				
11	1	1	1	**<0.001**	1	**0.007**	1	0.99	1	1			
12	**<0.001**	**<0.001**	**<0.001**	1	**0**	0.449	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**		
13	1	**0.01**	0.09	**0.02**	1	1	1	**<0.001**	1	1	1	**<0.001**	
14	0.10	**<0.001**	**<0.001**	1	1	1	0.17	**<0.001**	**0.01**	0.48	**0.02**	0.18	1

7.3.2 Betahydroxybutyrate

BOHB was only analysed for samples taken during the Adelaide assembly depot investigations (groups 10 to 14 listed above). Table 7-15 summarises the BOHB results. In sheep serum BOHB is normally below 0.7mmol/L (Barrington 1998).

Table 7-15 Serum BOHB results by group showing class, number of samples taken, mean for group and standard error (SE) of the mean with 95% CI

Assembly					Mean		95% CI	
Port	Depot	Group	Class	n	BOHB mmol/L	SE Mean	Low	High
Adelaide	**3**	**10**	Pastoral Wethers	50	0.23	0.01	0.211	0.253
		11	Lambs	33	0.28	0.01	0.257	0.310
		12	A Wethers	49	0.36	0.02	0.325	0.387
		13	Distance Wethers	49	0.20	0.01	0.174	0.218
		14	A Young Wethers	50	0.30	0.01	0.276	0.324
			Total	680	0.27	0.01	0.260	0.287

As with NEFA analysis, ANOVA was used to examine BOHB concentrations between groups of sheep. Results indicated a significant between groups effect (p<0.000) and follow-up tests were then conducted to compare mean values of BOHB between groups with Bonferoni adjustment to correct for multiple comparisons. Model checking included inspection of group specific variance estimates to check for homoscedasticity and inspection of a plot of standardised residuals vs. predicted values to check for normality of residuals and for evidence of any patterns. No problems were detected in these checks.

Table 7-16 Results of ANOVA for serum BOHB analysis. P-values from Bonferroni adjusted comparisons of BOHB concentrations for each of 5 different groups, performed as a series of follow-up tests after a one way ANOVA

Group ID	10	11	12	13
11	0.055			
12	**<0.001**	**0.001**		
13	0.483	**<0.001**	**<0.001**	
14	**0.002**	1.000	**0.022**	**<0.001**

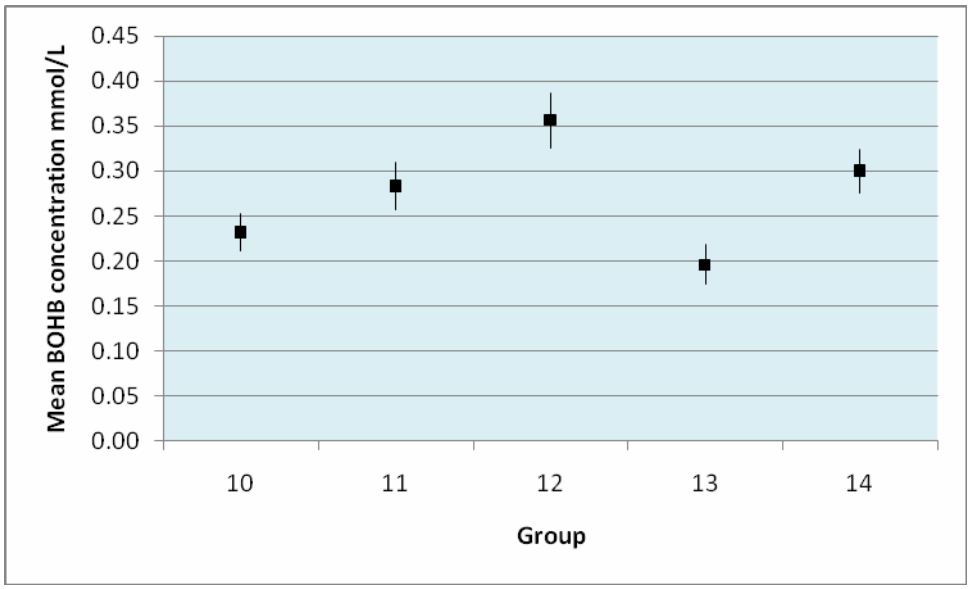


Figure 7-8 Average serum BOHB concentration by group with 95% CI

Table 7.16 shows the p-values for comparisons between groups, red values indicate that a significant difference exists between the two groups. The data are illustrated in figure 7-8. The analysis tells us that the average BOHB of group 12 is significantly higher than all other groups. Also average BOHB for group 14 is significantly higher than groups 10 and 13. The average BOHB concentration of group 12 (0.36mmol/L), the highest group in this study, was below the concentration reported for “non-feeding sheep” (0.7mmol/L) by Richards et al (1991).

7.3.3 Haptoglobin

Haptoglobin (Hp) is an acute phase protein produced in the liver. The acute phase response is a rapid change in the composition of certain blood proteins in response to infection or inflammation (Blood and Studdert 1999). Hp has been shown to be a sensitive indicator of acute bacterial infection and inflammation in sheep (Skinner and Roberts 1994). In normal ruminants, circulating hp is usually very low or undetectable, but increases over 100-fold on immune stimulation (Murata, Shimada et al. 2004). Investigations into hp in sheep have demonstrated that average hp for sheep groups with infectious disease are invariably greater than 1mg/ml (Aziz and Taha 1997). Several studies have demonstrated that hp concentrations peak 5-7 days after the infectious process begins and gradually decline to normal levels within 2-4 weeks (Pfeffer and Rogers 1989; Pfeffer, Rogers et al. 1993; Regassa and Noakes 1999).

Other studies have shown that hp can also increase following parturition, stress, road transportation and starvation in sheep and cattle (Murata and Miyamoto 1993; Arthington, Eicher et al. 2003). However, this effect is variable and has been demonstrated to occur in some groups of sheep and cattle and not others (Skinner, Brown et al. 1991; Arthington, Eicher et al. 2003). Furthermore, increases in hp following these events are less marked than in acute bacterial infection and have not been shown to exceed 0.5mg/ml (Yoshino, Katoh et al. 1993).

Hp was analysed on a subset of serum samples to determine if infection and inflammation were occurring in the assembly depot. Based on the information above it was determined all sheep with serum hp greater than 1.0mg/ml were suffering from an infectious disease process. A summary of serum samples analysed and haptoglobin results is given in table 7.17.

Table 7-17 Hp results by group showing class, number of samples taken, geometric mean for group and proportion (p) of samples greater than 1.0 mg/ml with 95% CI Need to shrink table a bit as doesn't print out properly, or put on landscape page.

Assembly					Mean#	95% CI					
Port	Depot	Group	Group ID	n	mg/ml	Low	High	n	p	Low	High
Portland	**1**	**2**	A Old Wethers	25	0.16	0.000	0.381	0	0.00	0.000	0.133
	2	**4**	Young Control	42	0.92	0.484	1.357	26	0.62	0.468	0.750
		5	Old Pastoral	18	0.16	0.000	0.683	2	0.11	0.031	0.328
		6	Control Wethers	38	0.21	0.000	0.457	2	0.05	0.015	0.173
		7	Young Pastoral	12	0.21	0.000	0.892	3	0.25	0.089	0.532
		9	B wethers	11	0.18	0.000	0.963	2	0.18	0.051	0.477
Adelaide	**3**	**10**	Pastoral Wethers	36	0.22	0.000	0.488	4	0.11	0.044	0.253
		12	A Wethers	27	0.22	0.000	0.473	1	0.04	0.007	0.183
		13	Distance Wethers	35	0.37	0.114	0.616	4	0.11	0.045	0.260
		14	A Young Wethers	35	0.36	0.075	0.645	3	0.09	0.030	0.224
			Total	279	0.29	0.161	0.419	47	0.17	0.129	0.217

Geometric mean - a geometric mean is used as haptoglobin results needed to be transformed to a natural log scale for analysis

Analysis of variance was used to examine haptoglobin concentrations between groups of sheep. Results indicated a significant between groups effect ($p<0.001$) and follow-up tests were then conducted to compare mean values of haptoglobin (on a natural log transformed scale) between groups with Bonferroni adjustment to correct for multiple comparisons. Model checking included inspection of group specific variance estimates to check for homoscedasticity and inspection of a plot of standardised residuals vs. predicted values to check for normality of residuals and for evidence of any patterns. No problems were detected in these checks.

Table 7-18 P-values from Bonferroni adjusted comparisons of log transformed Hp concentrations for each of 10 different groups. Performed as a series of follow-up tests after a one way ANOVA

Group	2	4	5	6	7	9	10	12	13
4	**<0.001**								
5	1.000	**<0.001**							
6	1.000	**<0.001**	1.000						
7	1.000	**<0.001**	1.000	1.000					
9	1.000	**<0.001**	1.000	1.000	1.000				
10	1.000	**<0.001**	1.000	1.000	1.000	1.000			
12	1.000	**<0.001**	1.000	1.000	1.000	1.000	1.000		
13	0.127	**0.002**	0.205	0.591	1.000	1.000	1.000	1.000	
14	0.151	**<0.001**	0.238	0.700	1.000	1.000	1.000	1.000	1.000

Table 7.18 shows the p-values for comparisons between groups, red values indicate that a significant difference exists between the two groups. The analysis tells us that the average haptoglobin for group 4 is significantly higher than all other groups. There are no other significant differences between groups. This data is displayed in figure 7.9.

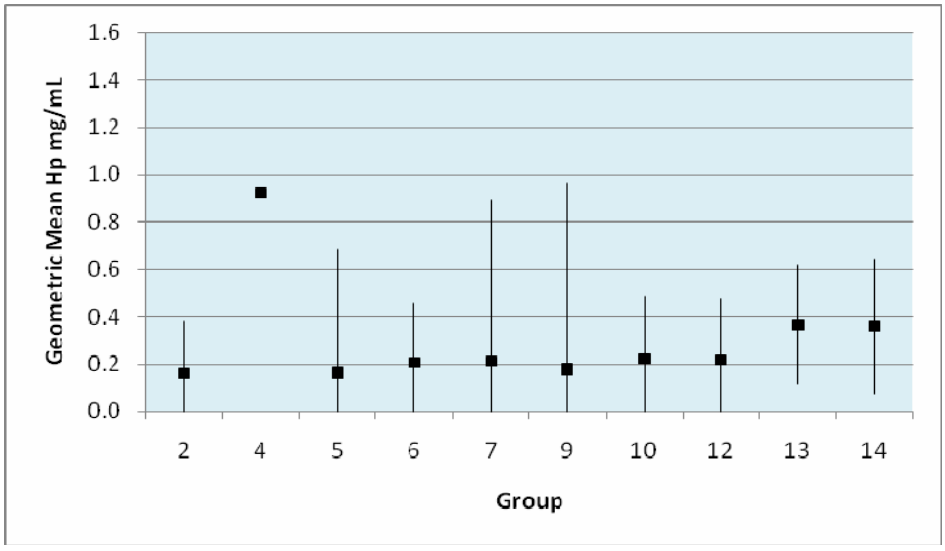


Figure 7-9 Geometric mean of serum haptoglobin concentration by group with 95% CI

7.4 Discussion

7.4.1 Limitations of investigation

These results along with on ship post-mortem examinations can potentially be used to determine the distribution and spread of salmonella organisms within the assembly depot and the importance of this in the development of ovine salmonellosis. Our ability to interpret these and other relationships between events in the assembly depot and outcome on ship is limited because the identity of the sheep in each paddock is unknown.

On receival at the assembly depot sheep are drafted into classes based on sex, breed, type, and weight. Sheep from a single vendor may be drafted into different classes and the identity of sheep in each class and paddock is not recorded. When sheep are loaded out of the assembly depot for loading on ship paddock groups are not maintained so it is also not possible to evaluate outcome based on assembly paddock.

As paddock groups cannot be followed beyond the assembly depot, it has also not been possible to assess if sheep from paddocks with higher salmonella contamination or with higher faecal salmonella shedding have a greater risk of salmonellosis and mortality during the voyage. The only groups that are directly traceable in the assembly depot and during the voyage are research lines. These sheep are not classed and mixed at receival and remain together during the assembly period and voyage. For other groups, we can only compare these results to on ship performance of all sheep of the same class. For example, we do not have specific information of the on-ship performance of group 12 but we do have information on the performance of all A-wethers loaded in Adelaide.

7.4.2 Summary of findings

The prevalence of shedding was low with approximately 3% of sheep shedding salmonella at the time of sampling

Previous studies have demonstrated faecal salmonella shedding at the end of the assembly period is highly variable, ranging from less than 1% to as high as 83% (Higgs, Norris et al. 1993; Kelly 1996). Faecal salmonella shedding has been shown to be close to zero on farm and at the time of receival, however shedding increases during the assembly period. These sheep were sampled towards the end of the assembly period (1 and 2 days before load out); a faecal salmonella shedding prevalence of 3% at this stage can be considered to be low. The subsequent mortality rate for sheep loaded from these ports for these voyages was 0.54% and 1 % for the Portland and Adelaide loaded sheep respectively.

Although 90% of environmental samples were negative for salmonella, environmental salmonella contamination increases during the assembly period

No Salmonella were isolated from 60% and 50% of the paddocks sampled in the Portland and Adelaide investigations respectively. The salmonella studies detailed above demonstrated that environmental salmonella contamination increases during the assembly period and that this can be associated with excretion of potentially virulent salmonella organisms in faeces.

Pathogenic salmonella serotypes that were present in the assembly depot were found to cause clinical salmonellosis and mortality during the voyage
Salmonella organisms can persist in the environment for long periods and rapidly proliferate under favourable environmental conditions

It is anticipated that the number of salmonella present in the environment will be affected by several factors. Salmonella will proliferate under favourable conditions of moisture and temperature and be destroyed by extreme heat and cold or by desiccation (drying out). Faecal matter can increase environmental salmonella contamination directly; through faecal salmonella shedding and indirectly; as faeces provide a nutrient media within which salmonella may proliferate. From a sheep management perspective it is important to appreciate that the environment provides a dynamic reservoir of infection. Salmonella challenge is a function of both salmonella numbers and virulence. Sheep entering the feedlot at different times of the year would be expected to be challenged with different numbers of salmonella. With greater challenge more likely following high sheep throughput and following moist conditions.

The yards are an important point for exposure to salmonella organisms

As discussed above, faecal salmonella shedding is highest at the end of the assembly period; this may result in the yards becoming heavily contaminated during load out and provide a reservoir of infection for the next consignment of sheep received. The role yards play in exposing sheep to salmonella is more clearly demonstrated in the West Australian assembly depots where sheep are housed in sheds with slatted floors. The sheds provide a relatively manure free environment. Although sheep from different sheds have no direct contact, during an outbreak of salmonellosis, disease was observed across multiple sheds. The only common exposure source are the yards, all sheep pass through the yards on their way to the sheds and during the assembly period mobs of sheep experiencing problems are redrafted in the same yards to remove sick sheep.

Antimicrobial resistance is not common in live export salmonella isolates

In regards to therapeutic management of sick sheep on ship, different approaches were employed by different shipboard veterinarians, these included no treatment, feeding chaff, injecting individual sheep with tetracycline, and pen medication with tetracycline. It was not clear which approach was most efficacious and opinions differed suggesting a need for investigation and establishing a logical decision making process.

The proportion of sheep with serum NEFA >1mmol/L was 0.33 (95% CI 0.294, 0.364). This indicates that 33% of sheep sampled were mobilising fat reserves

None of the samples analysed for serum BOHB concentration were outside the reference range of <0.7mmol/L

The results of the NEFA and BOHB investigation indicate a lower proportion of sheep experienced profound negative energy balance than was reported in previous investigations. (Richards, Hyder et al. 1991) The current investigations were conducted in September and August which corresponds to the same time of year as the previous study (Richards, Hyder et al. 1991).

The proportion of sheep with serum haptoglobin >1.0mg/ml was 0.17 (95% CI 0.129, 0.217). This indicates that 17% of sheep sampled were experiencing an acute phase response Group 4 stands out with more than half of the samples greater than 1.0mg/ml coming from this group

7.4.3 Interpretation of results by group

Table 7-19 summarises the environmental and faecal salmonella culture results as well as blood biochemistry results for sheep groups which had significant results.

Table 7-19 Summary of salmonella culture and serum biochemistry results for all groups with significant results

Port	Assembly Depot	Sheep Group	Class	Salmonella Culture		Serum Biochemistry	
				Faecal	Paddock	NEFA/BOHB	Haptoglobin
Portland	**2**	**4**	Young Non-pastoral Wethers	High	High	High	High
		7	Young Pastoral Wethers	Low	High	Normal	Not Sampled
		6	Old Non Pastoral	Low	Low	High	Normal
Adelaide	**3**	**12**	A Wethers	High	High	High	Normal
		13	Distance Wethers	Low	Low	Normal	Normal
		14	A Young Wethers	Low	Low	High	Normal

Group 4 developed salmonellosis which led to inappetence and negative energy balance

This control group had significantly higher average NEFA concentrations than all other groups excluding groups 6 and 12. In Addition to this, mean haptoglobin for the group was significantly higher than all other groups. This control group also had the highest proportion of salmonella positive faecal cultures, with 100% of the pooled faecal cultures positive for salmonella. This shedding contributed to an increase in the proportion of salmonella positive environmental cultures for this paddock.

The finding of elevated haptoglobin in the young wethers suggests this group was experiencing an infectious disease process. During the sampling process it was noted that animals in this group had diarrhoea and a large proportion were subsequently determined to be shedding salmonella. Taken together the data suggest that this group was experiencing clinical salmonellosis. Young sheep normally have a strong appetite drive; the negative energy balance in this group appears most likely to reflect the culmination of reduced appetite secondary to disease and an elevated metabolic rate induced by salmonellosis.

Group 7 was housed with group 4 in a paddock with heavy salmonella contamination but did not have any evidence of negative energy balance or salmonella infection

For this young pastoral wether group (Group 7), average NEFA concentration and proportion of salmonella positive faecal cultures were not significantly higher than any other group. This group was definitely exposed to salmonella in the environment but were not inappetent and did not shed salmonella in their faeces.

Sheep from group 4 were penned with group 7 on ship and neither group experienced high mortality

Groups 4 and 7 were housed in the same paddock, entered the assembly depot and paddock at the same time and were likely exposed to similar salmonella challenge. The evidence indicates that group 4 experienced significantly higher levels of negative energy balance and salmonellosis than group 7. The evidence does not specify if negative energy balance preceded salmonellosis or vice versa, nor does it explain the different outcomes for sheep in group 4 and 7. These differences may have occurred due to a higher proportion of group 4 failing to eat in the first couple of days of the assembly period leading to animals in this group becoming more susceptible to salmonella infection and developing salmonellosis. Conversely, it may reflect that a higher proportion of animals in this group had lower immunity to salmonella on arrival and subsequently developed salmonellosis leading to mobilisation of fat reserves in response to disease induced anorexia and catabolism.

The timing of sampling gives us some indication of which disease process occurred first. All samples were collected 6 days after sheep were received at the assembly depot. Hp has been shown to peak 5-7 days after initial infection. The true mean Hp concentration for groups 4 and 7 were 1.8mg/ml and 0.48mg/ml respectively. As a single sampling was performed, the exact duration of Hp elevation is not certain, however, the magnitude of Hp elevation seen in group 4 suggests that infection and inflammation have been present for several days. Elevated serum Hp six days after receival suggests that salmonella challenge occurred close to the time of receival. In addition to this, young sheep have been shown to have a strong appetite drive, therefore it is less likely that sheep in this group experienced voluntary refusal to eat. We suspect that for this group salmonellosis was the initiating disease process leading to depression of appetite and subsequently negative energy balance.

Group 12 had a high proportion of sheep (86%) in negative energy balance, high faecal salmonella shedding but no evidence of active inflammation

This group of sheep (Group 12) had significantly higher average serum NEFA concentrations than all other groups excluding groups 4 and 6 and higher serum BOHB concentrations than all other groups that were sampled. This group also had a high proportion of positive faecal salmonella cultures. The proportion of salmonella positive environmental cultures for this paddock increased during the assembly period and was

higher than all other groups. Average serum haptoglobin for this group was not significantly higher than any other group. In this group it is likely that inappetence was the initiating disease process leading to negative energy balance and increased salmonella colonisation in the gut and faecal salmonella shedding. The normal haptoglobin concentrations suggest that either the salmonella infections were not invasive or that the timing of exposure to salmonella was later in the assembly period and the sheep had not yet mounted a significant inflammatory response. Mortality in A wethers loaded from Adelaide (of which group 12 are a subset) experienced 2.2% mortality. The A wethers accounted for 14% of the sheep loaded and for 30% of the mortality for sheep loaded in Adelaide. Enteritis accounted for the highest percentage of diagnoses for these sheep.

Groups 6 and 14 had a high proportion of sheep in negative energy balance but did not shed salmonellae in faeces or have evidence of inflammation

Both of these groups (Groups 6 and 14) demonstrated negative energy balance without faecal salmonella shedding. Serum NEFA for both groups was significantly higher than several other groups and the group averages (1.06mmol/L and 1.00 mmol/L) indicate that inappetence is occurring. Low numbers of salmonella were recovered from the group 6 paddock but no salmonella were isolated from the group 14 paddock. These groups had on ship mortality less than 1%.

Group 13 had no evidence of negative energy balance, inflammation or salmonella shedding One line within this group experienced a salmonellosis outbreak during the voyage suffering 22.6% mortality (95/420)

The average serum NEFA, BOHB and haptoglobin for Group 13 were not significantly higher than any other group. Average NEFA and BOHB were both below the normal reference range (0.65mmol/L and 0.3mmol/L respectively). Average haptoglobin was not above the normal reference range. There was a low prevalence of faecal salmonella shedding in this group and low levels of salmonella in the environment.

Group 13 went on to experience salmonellosis mortality from day 8 of the voyage (10 days after sampling) which led to inappetence and inanition in the final stages of the journey. While there was no evidence of inflammation during the assembly period, salmonellosis on ship followed an incident where the ship pen became wet, which likely allowed salmonella organisms to proliferate and increased salmonella challenge. The salmonellosis was largely confined to one line of sheep which experienced 22.6% mortality (95/420). It is possible that this line had a lower level of immunity to infection. Overall mortality for distance wethers on this voyage was 6.0% (98/1634).

The results of these more intensive investigations illustrate the complexity of the associations and interactions between inappetence, salmonellosis and mortality. Some lines of inappetent sheep were observed to have higher mortality whereas others did not. In one group of sheep inappetence appears to have been a risk factor for salmonella shedding and in another salmonella infection appears to have contributed to inappetence. Salmonella faecal shedding was observed to be associated with inflammation and disease in some sheep and not in others. High mortality associated with salmonellosis on ship was observed in sheep that were neither demonstrated to be in negative energy balance or shedding salmonella during the assembly period.

7.5 Shipboard observations of feed intake

The results of postmortem examinations suggest that the current export pellet ration is of good quality. Despite evidence of negative energy balance occurring in the assembly depot, the overall occurrence of inanition was found to be lower than reported in previous studies (accounting for 23.1% of mortalities rather than the 43.4% reported by (Richards, Norris et al. 1989)) indicating that fewer sheep are failing to adapt to the ration. In addition to this, there was no evidence of gastrointestinal disease related to the ration, more than 5,600 post-mortem examinations were performed during investigation, and no cases of ruminal acidosis were recorded.

Pellet dust was observed to accumulate in feed troughs to a greater or lesser extent during different voyages. The policy of the ships chief officer appeared to be a driving factor in this area. The ships Chief Officer is responsible for fodder management during the voyage. Ruminants generally do not like dusty feed and intakes will be favoured by avoiding the build up of pellet dust.

In regard to inappetence on the ship it was noted that the feeders are positioned such that the sheep have to put their head through to the outside of the pen. In cattle feedlots the concept of shy feeders is recognised and facilities often include stanchions to cater for shy feeders by providing less intimidating access to feed.

Dry matter intake is also influenced by access to water. If water is limiting livestock tend to eat less. Cleaning of water and feed troughs is part of the daily routine on ships. From a labour perspective cleaning troughs is relatively labour intensive. Crew often switch off the waters during the evening to facilitate cleaning first thing in the morning. Leaking troughs can be problematic as they cause wetting of the faecal pack and need to be switched off.

7.6 Causal pathways of disease

7.6.1 Salmonellosis

Salmonellosis refers to the clinical disease associated with salmonella infection. The causal pathways for development of salmonellosis are shown in figure 7.10. The common clinical signs of salmonellosis in sheep include anorexia, fever, diarrhoea, depressed mentation and death. Salmonella are relatively uncommon in extensive animal production systems, with the prevalence increasing with intensification and increasing herd size (Radostits, Gay et al. 2000). Higgs et.al. (1993) found that on arrival at Australian live export assembly depots the prevalence of faecal salmonella shedding is close to zero, ranging from 0% to 0.70%.

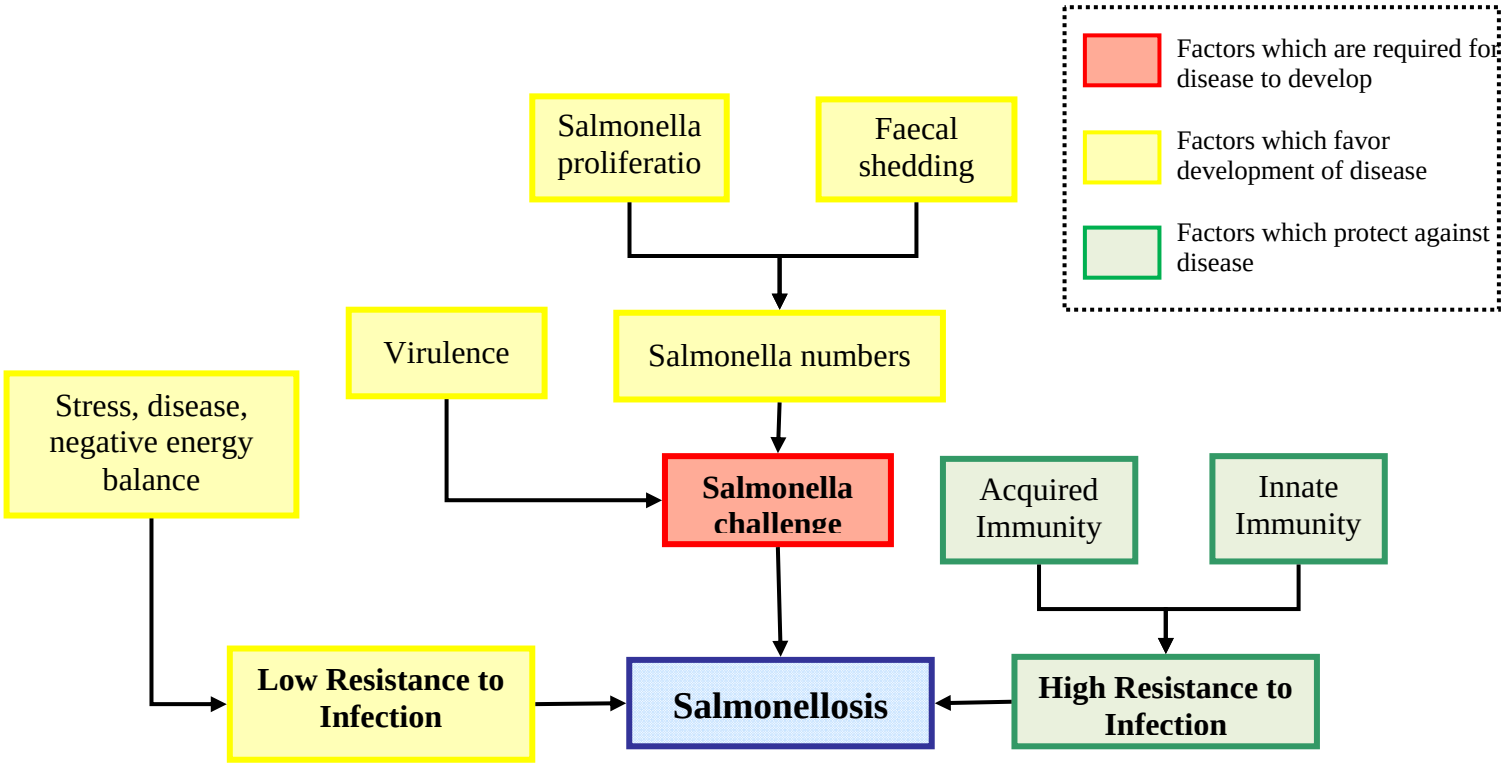


Figure 7-10 Causal pathways for development of salmonellosis

Several factors are important in the development of salmonellosis. These include salmonella challenge (number of bacteria and virulence of the strain) and specific immunity to salmonella infection (influenced by previous exposure to salmonella) as well as general state of health and resistance to infection (influenced by concurrent disease, nutrition and stress). Ingestion is thought to be the most common route of infection. Ingested bacteria can enter the host via the tonsils or pass through to the forestomachs and enter the host via the intestine. Experimental studies in calves and piglets in which animals were oesophagectomised have demonstrated that salmonella distribute throughout the body within hours of ingestion even when they are prevented from passing through the gastrointestinal tract (De Jong and Ekdahl 1965; Fedorka-Cray, Kelley et al. 1995). Other experimental salmonella challenge studies have illustrated a challenge dose response. Hundreds of salmonella may be sufficient to cause disease in compromised sheep where as 10^9 may be required to cause mortality in healthy sheep (Wray and Linklater 2000). The growth of salmonella in the rumen following ingestion is influenced by dietary intake before and after the organisms are ingested (Brownlie and Grau 1967). The growth of salmonella in the rumen is inhibited by high concentrations of volatile fatty acids and a low rumen pH (normal is 5.5-6.5). (Chambers and Lysons 1979; Mattila, Frost et al. 1988). Anorexia is associated with low concentrations of volatile fatty acids and a high rumen pH (approaching pH 7.5). Salmonella disappear rapidly from the rumen of regularly fed cows, but maintain or increase their numbers when feed intake is decreased or interrupted for one or more days (Brownlie and Grau 1967). Feeding after a period of starvation is associated with multiplication of Salmonella (Grau, Brownlie et al. 1968; Frost, O'Boyle et al. 1988). Clinical and subclinical lactic acidosis increases the risk of salmonellosis as disruption of normal fermentation, with production of lactate, favours the less fastidious salmonella, which multiplies rapidly using the available substrate (Chambers and Lysons 1979).

Low dose salmonella challenge that does not overwhelm the host is capable of stimulating protective immunity. The timing and manifestations of disease following salmonella challenge are influenced by the virulence of the infective strain, the challenge dose and host immunity. Environmental conditions often impact the host pathogen interaction by altering the magnitude of the challenge dose (moisture) and through impacting host immunity (heat or cold stress). According to experimental challenge experiments in calves where the challenge dose and timing of infection are known fever is often observed within 36 – 48 hours, depression of appetite by 48 hours and diarrhoea within 48 – 72 hours (Dueger et. Al. 2003, Fecteau et al. 2003, Mohler et al. 2006, Mohler et al 2008) A large challenge dose produces a rapid onset of clinical signs and a lower dose a slower onset of clinical signs reflecting the pathogenesis of the disease which includes bacterial elimination and multiplication phases. Different salmonella strains differ in invasiveness and propensity to cause bacteraemia and diarrhoea. The disease usually runs its course over 14 – 21 days; it is uncommon for animals to die after 14 days unless they experience a concurrent compromise. In a commercial setting the timing of pathogen exposure and the immunity of the population will be variable providing for greater spread in the timing and manifestations of disease. Chronic salmonella infections are rare, most often associated with host adapted serotypes. The host adapted salmonella serotype for sheep, *Salmonella abortus ovis* has never been reported in Australia.

Salmonella can survive for months to years in the environment and under suitable conditions will proliferate to increase the level of environmental contamination (Murray 2000). In live export pre-export assembly depots, *Salmonella typhimurium and Salmonella bovismorbificans* are frequently isolated from different consignments, suggesting environmental persistence between consignments with sheep becoming infected during the assembly period and on ship (Kelly 1996).

7.6.2 Inappetence, negative energy balance and inanition

The causal pathways for inappetence, negative energy balance and inanition are shown in figure 7.11. Inappetence is defined as lack of appetite (Blood and Studdert 1999). Inappetence leads to negative energy balance. Negative energy balance can lead to ketosis and inanition. Inanition is defined as an exhausted state of prolonged under nutrition or starvation (Blood and Studdert 1999). Inappetence in ruminants may be caused by increased body temperature, toxaemia, cobalt deficiency, parasitic burdens, pain or fear. In addition to this it has been shown that some pasture fed sheep become completely inappetent when housed and hand fed (Radostits, Gay et al. 2000). There are numerous variables that could contribute to sheep inappetence in the live sheep trade. A study by Norris et al. (1989) found that the percentage of non-feeders at the end of the assembly period ranged from 0.2% to 23.3%. However, most assembly depot non-feeders will begin to eat early during the ocean voyage, with 82.4% of assembly depot non-feeders eating by day 5 of the voyage and the number of non-feeders continuing to steadily decrease throughout the voyage (Norris, McDonald et al. 1990). Despite the fact that a majority of assembly depot non-feeders will eventually begin to eat during the voyage they remain more likely to die during the export process.

7.6.3 Relationships

The relationships between inappetence, salmonellosis and inanition are shown in figure 7.12.

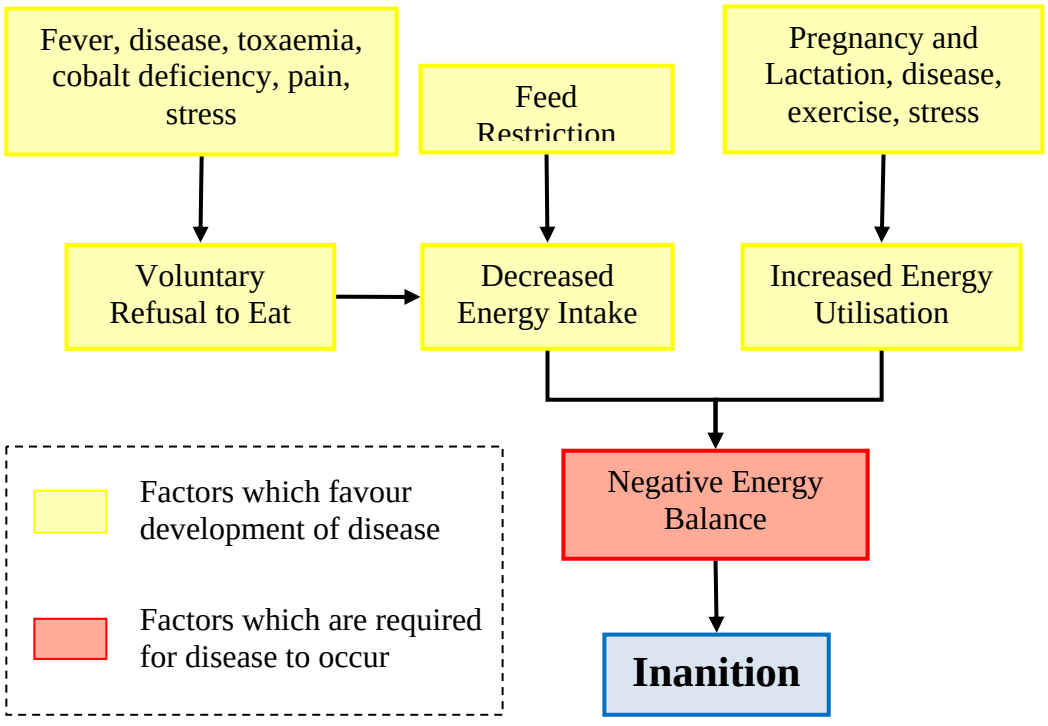


Figure 7-11 Causal pathways for inappetence, negative energy balance and inanition

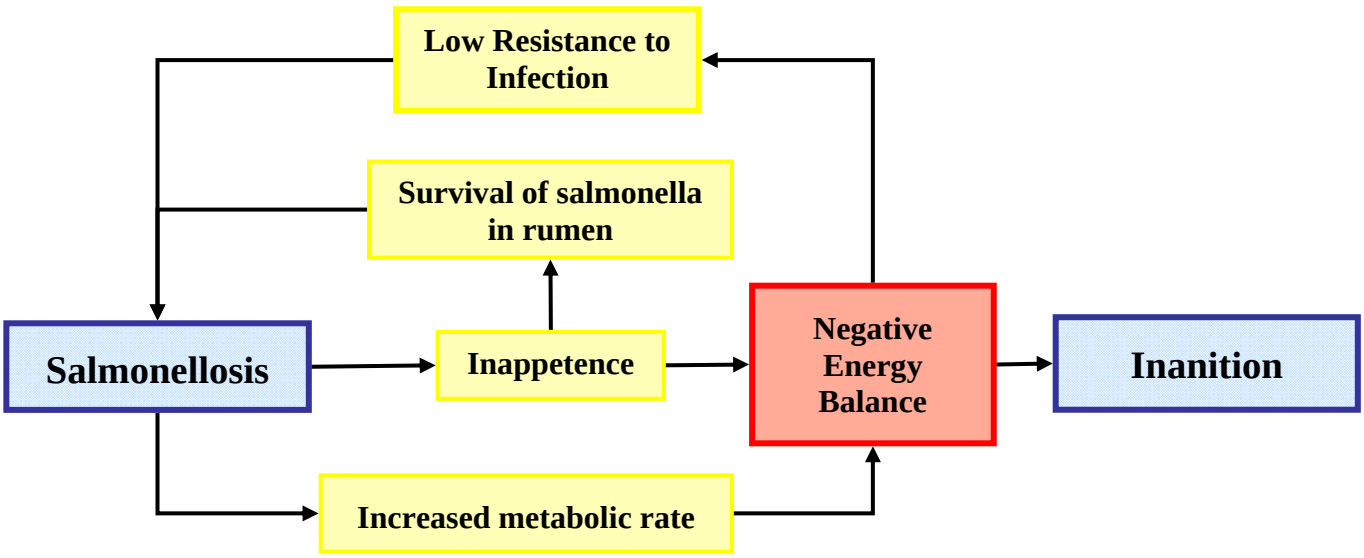


Figure 7-12 The relationships between inappetence, salmonellosis and inanition

Salmonellosis Secondary to Negative energy Balance
The rumen environment is adverse to salmonella in healthy ruminants that are eating normally. Decreased feed intake results in changes to the constitution and pH of rumen fluid, allowing salmonella organisms to survive and potentially proliferate. As a result, more salmonella organisms are permitted to pass into the intestine where they can potentially cause disease (Brownlie and Grau 1967). However, when feeding resumes, salmonella are rapidly eliminated from the rumen. Negative energy balance and ketosis have also been associated with suppression of the immune system and increases susceptibility to bacterial infections (Radostits 2000).

Salmonellosis and inanition remain the most common causes of mortality in the live sheep trade (see section 9.2 for details). However, over the past 10 years, the demographic of the exported flock has changed with more young sheep and fewer older heavy wethers entering affect the development of disease (such as host immunity, appetite drive and level of salmonella exposure) are continually fluctuating.

8 Voyage mortality – statistical analysis

8.1 Materials and methods

8.1.1 Data collection

Shipboard data was collected for 27 voyages. During the voyage, mortality data was collected by members of the project team and AQIS shipboard veterinarians. A total of 5 voyages (voyages 1, 4, 9, 17 and 22) were accompanied by a member of the project team. Data were collected during the daily mortality rounds. Data collected included:

- Location (i.e. row section and where applicable tier)
- Class of sheep
- Property of origin identification, primarily ear tag
- Port of loading and pre-export assembly depot

In addition to this post-mortem examinations were carried out on a subset of mortalities and are discussed in detail under section 9.

8.1.2 Statistical analysis

The final data set contained one row for each line of sheep loaded onto the export vessel. Categorical variables in the final data set included age, sex, breed, origin variables (state, pastoral), voyage, ship, month, year, assembly depot, and death and rejection on arrival. Body weight, curfew time, and weather data for the assembly depot and property of origin were stored and analysed on a continuous scale. Distance travelled to assembly depot and duration of assembly period was classified into categories for analysis.

Descriptive statistics were performed for the outcome variables and each of the predictor variables. For analysis of mortality during the voyage negative binomial regression was used. Negative binomial regression is a special form of logistic regression for count data that are heavily clustered. In both analyses the outcome variable was a count and the data were heavily clustered. The output of negative binomial regression is a relative risk. Relative risks are used to compare incidence rates; if the p-value is less than 0.05 then the difference between groups is significant.

Univariate screening was performed for all variables of interest in the data set. The scale of the continuous predictors was checked to determine if they had a linear relationship with the outcome variable. The predictor variables were correlated to ensure that highly correlated variables were not included in the final model. The model was built using a backwards manual stepwise approach to produce a final main effects model containing only significant variables. The excluded predictor variables were added back to the model one by one to ensure that significant variables were not omitted and to check for confounding. Biologically plausible interaction terms were considered. Model checking was by assessing overall fit and residuals as described by Dahoo (Dohoo, Martin et al. 2003).

8.2 Results

8.2.1 Traceability of mortality

For the shipboard mortality investigations the analysis was limited to consignments from ports where more than 50% of the mortality could be accounted for. Data were excluded if the receival information or ship mortality information did not allow more than 50% of voyage mortality to be accurately traced back to a receival. As such, data were excluded in groups, i.e. all data from one receival, for one voyage, at one port of loading. The average traceability of the voyages included in the analysis was 69.82%. Tables 8.1 to 8.3 show number of sheep accepted, mortality and traceability of mortality for the voyages studied by port. The final database included data from 24 voyages, comprising 13 consignments assembled and loaded in Portland, 10 consignments assembled and loaded in Adelaide and 12 consignments assembled and loaded in Fremantle.

A significant difference was observed in the traceability of mortality for sheep loaded from different ports. Traceability was very good for sheep loaded from Portland (84.5%), good for Adelaide (78.0%) and variable for sheep loaded from Fremantle (56.9%).

Table 8-1 Sheep accepted, mortality and mortality traceability by voyage for sheep assembled and loaded in Fremantle

Voyage	Sheep Accepted	Mortality Count	Percentage Mortality	Number Traced	Percentage Traced
1	64,080	927	1.45%	754	81.3%
2	19,533	253	1.30%	45	17.8%
3	12,572	56	0.45%	0	0.0%
4	45,703	293	0.64%	143	48.8%
6	46,647	341	0.73%	253	74.2%
7	72,141	191	0.26%	82	42.9%
8	55,162	572	1.04%	321	56.1%
10	41,925	333	0.79%	207	62.2%
13	55,736	462	0.83%	316	68.4%
14	22,633	87	0.38%	52	59.8%
15	41,454	154	0.37%	128	83.1%
16	44,852	260	0.58%	93	35.8%
17	58,045	1,011	1.74%	255	25.2%
18	72,595	284	0.39%	204	71.8%
19	58,590	497	0.85%	278	55.9%
20	69,497	1,127	1.62%	856	76.0%
22	45,019	291	0.65%	194	66.7%
23	71,309	649	0.91%	425	65.5%
25	78,727	524	0.67%	127	24.2%
Total	**976,220**	**8,312**	**0.85%**	**4733**	**56.9%**

Table 8-2 Sheep accepted, mortality and mortality traceability by voyage for sheep assembled and loaded in Portland

Voyage	Sheep Accepted	Mortality Count	Percentage Mortality	Number Traced	Percentage Traced
1	41,482	227	0.55%	223	98.2%
2	55,137	359	0.65%	329	91.6%
3	59,942	466	0.78%	421	90.3%
4	55,000	318	0.58%	288	90.6%
6	32,401	205	0.63%	153	74.6%
7	32,976	123	0.37%	110	89.4%
9	34,620	420	1.21%	328	78.1%
10	34,029	357	1.05%	286	80.1%
14	48,962	404	0.83%	365	90.3%
16	19,050	111	0.58%	103	92.8%
19	25,668	222	0.86%	138	62.2%
21	37,235	235	0.63%	215	91.5%
23	56,906	342	0.60%	241	70.5%
Total	**533,408**	**3,789**	**0.71%**	**3200**	**84.5%**

Table 8-3 Sheep accepted, mortality and mortality traceability by voyage for sheep assembled and loaded in Adelaide

Voyage	Sheep Accepted	Mortality Count	Percentage Mortality	Number Traced	Percentage Traced
5	74,704	518	0.69%	234	45.2%
9	38,096	391	1.03%	334	85.4%
11	76,702	883	1.15%	711	80.5%
12	70,431	655	0.93%	556	84.9%
15	40,128	246	0.61%	211	85.8%
17	28,220	600	2.13%	509	84.8%
18	14,480	74	0.51%	52	70.3%
21	65,379	1,258	1.92%	1166	92.7%
22	69,440	673	0.97%	609	90.5%
24	76,453	710	0.93%	326	45.9%
38	52,183	275	0.53%	201	73.1%
39	18,408	38	0.21%	20	52.6%
Total	**554,033**	**6,321**	**1.14%**	**4929**	**78.0%**

8.2.2 Summary statistics

8.2.2.1 Mortality by voyage

Mortality was seen to vary between voyages. Figure 8.1 shows the overall percentage mortality with 95% confidence intervals for each voyage. This variation is explored in the multivariate analysis below.

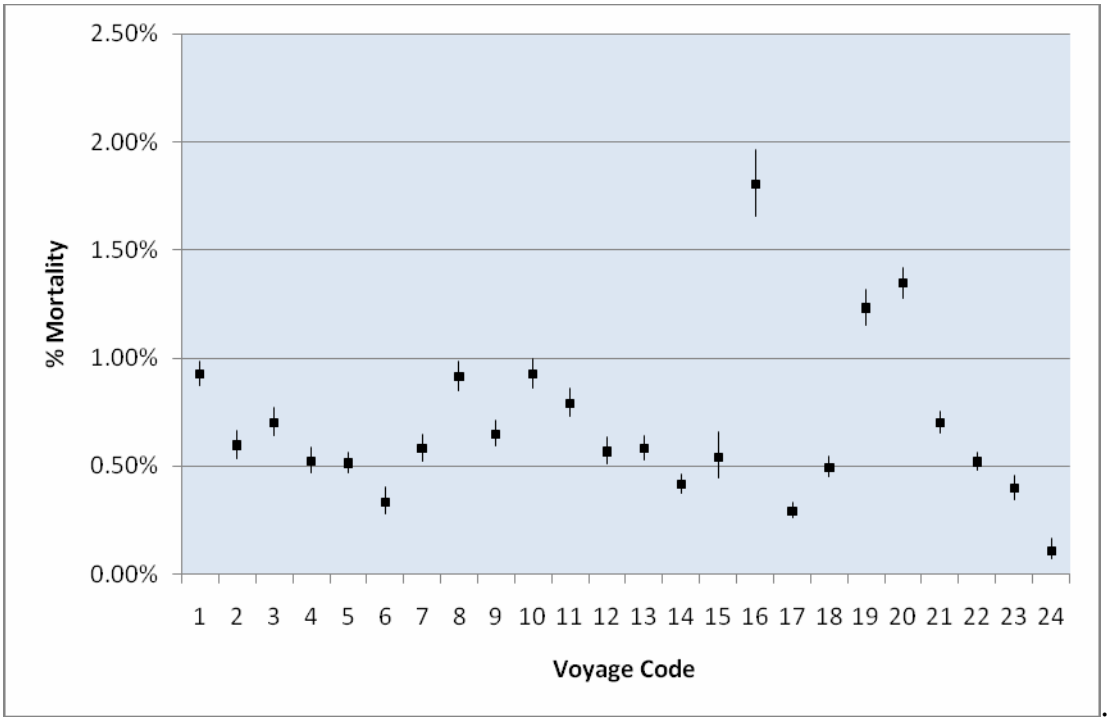


Figure 8-1 Percentage mortality with 95% CI for each of the 24 voyages included in the final analysis

8.2.2.2 Mortality by line

There were 8,201 lines of sheep in the final database. Of these 5,144 (62.7%) had no recorded sheep mortalities. 4 lines had 100% mortality; however these lines consisted of 1 or 2 sheep. Of the 3,057 lines that had recorded mortality, 1,549 (50.7%) had a mortality rate of 1% or less, 74% of all mortality was traced to 18% of lines. Figure 8.2 shows the proportion mortality for each line of sheep with 95% confidence intervals for all lines of greater than 50 sheep that had mortality arranged in order of increasing proportion mortality. The figure demonstrates the variation in mortality by line.

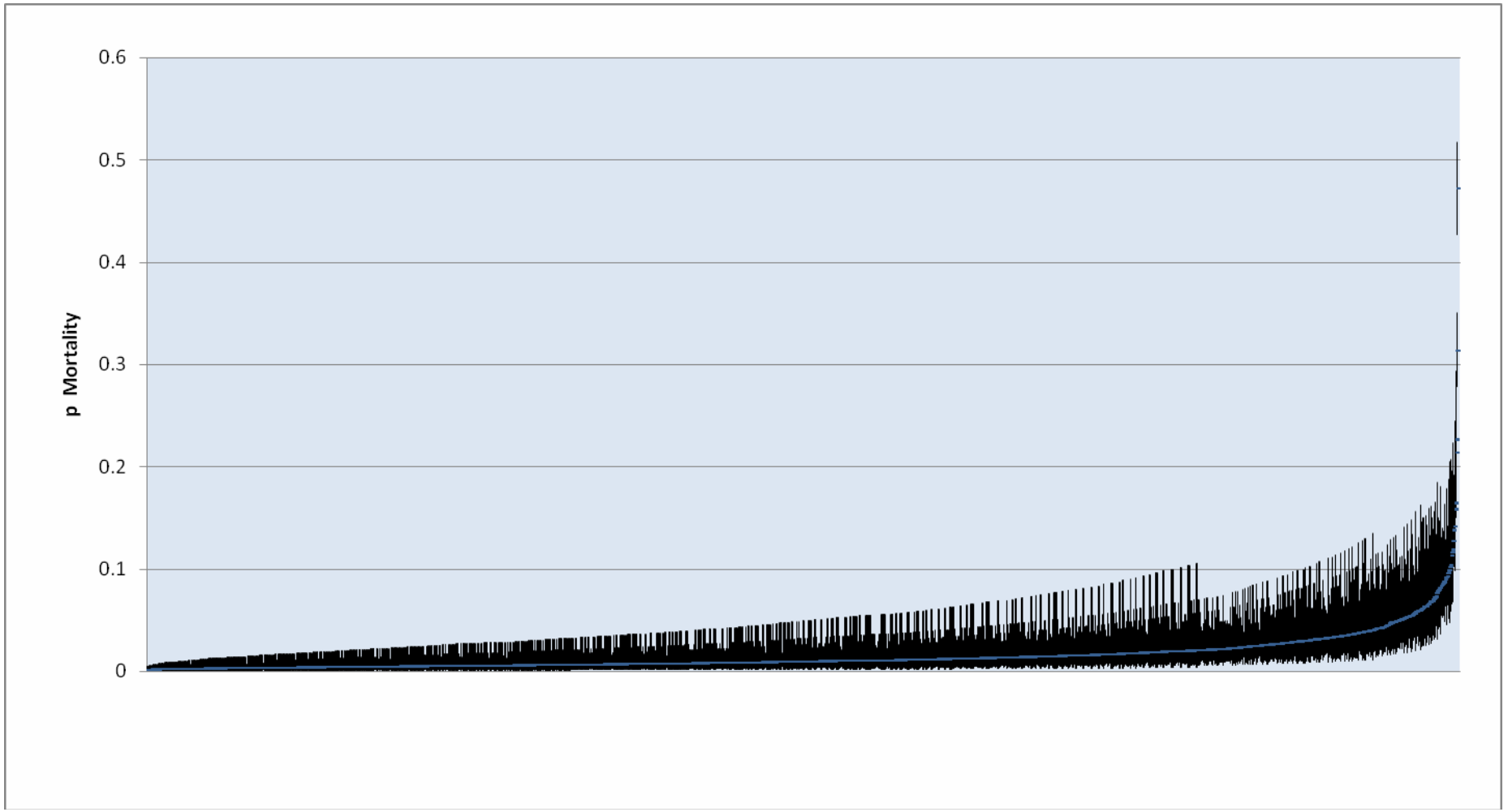


Figure 8-2 Caterpillar plot of proportion mortality and 95% CI for all consignments of more than 50 sheep that had mortality arranged in increasing order

8.2.3 Summary of research lines

Research lines included sheep from the pastoral zone, distance sheep (sheep travelling more than 800km to reach the assembly depot) and lambs assembled in either Adelaide or Portland between May and October each year.

8.2.3.1 Distance sheep

Summaries are shown below for distance sheep, lambs, pastoral sheep from South Australia and pastoral sheep from New South Wales.

Table 8-4 Summary statistics for distance sheep including voyage code, line code, number accepted, mortality count and mortality % with 95% confidence intervals.

					95% CI	
Voyage	Line	n	Mortality Count	Mortality %	Lower	Upper
9	1	420	95	22.62%	18.88%	26.86%
9	2	614	0	0.00%	0.00%	0.62%
9	3	580	3	0.52%	0.18%	1.51%
Total		**1614**	**98**	**6.07%**	**5.01%**	**7.34%**

Due to the high mortality for line 1 the history of these sheep was further investigated. The sheep were purchased from Wagga Wagga sale yard by a Victorian vendor. The sheep were held in Victoria for a period longer than 30 days before being delivered to the registered premises in Adelaide. As such the management of these sheep conformed with the export standards of the time and as such the sheep did not qualify as a “research” line. As distance sheep were only exported on one voyage (due to changes in the Export Standards) and there were fewer than 2000 animals in total they were not included in the multivariate analysis, instead distance travelled to reach the assembly depot was included in the analysis for all sheep.

8.2.3.2 Lambs

As with the distance sheep, lambs were only included in one voyage and there were fewer than 2000 animals in total.

Table-8-5 Summary statistics for lambs including voyage code, line code, number accepted, mortality count and mortality % with 95% confidence intervals

					95% CI	
Voyage	Line	n	Mortality Count	Mortality %	Lower	Upper
9	1	1998	1	0.05%	0.01%	0.28%

These lambs were not included as a separate group in the multivariate analysis. A general age category was included in the multivariate analysis and the research line of lambs was grouped together with all other lambs.

8.2.3.3 Pastoral sheep

Figure 8.3 shows the pastoral zones of Australia. For the current project, sheep were sourced from the pastoral zone of both South Australia and New South Wales. Sheep sourced from north of Port Augusta in South Australia and sheep sourced from the western districts of NSW were classified as pastoral.

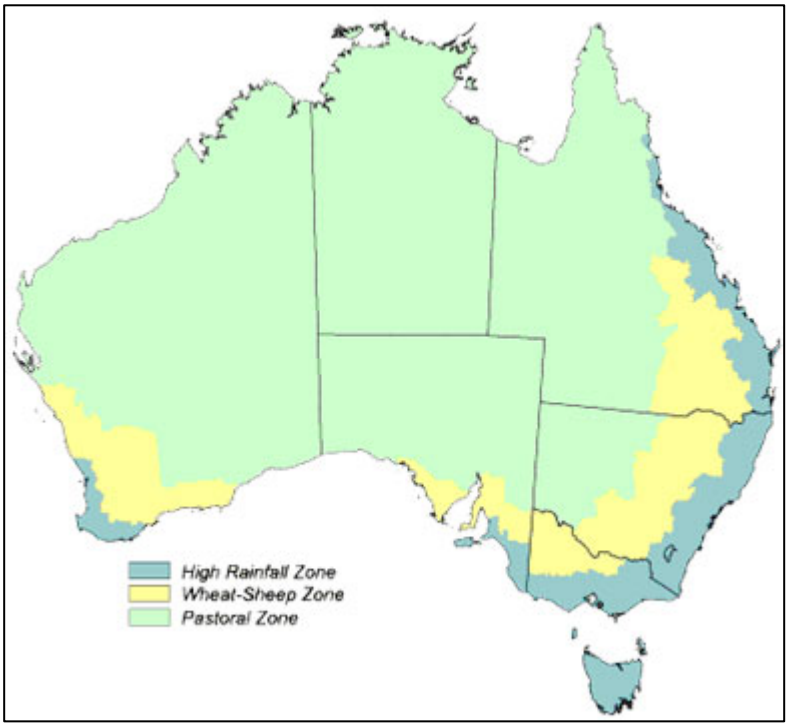


Figure 8-3 The pastoral zones of Australia

There were 17 lines of pastoral sheep from SouthpAustralia exported on 4 separate voyages. Average mortality for pastoral sheep from South Australia was 1.02% (0.85%, 1.22%). There were 2 lines in which mortality exceeded 2%, which is the reportable mortality level set out in the Australian standards for the export of livestock.

Table 8-6 Summary statistics for pastoral sheep from South Australia including voyage code, line code, number accepted, mortality count and mortality % with 95% confidence intervals

					95% CI	
Voyage	Line	n	Mortality Count	Mortality %	Lower	Upper
9	1	391	2	0.51%	0.14%	1.85%
9	2	3	0	0.00%	0.00%	56.15%
9	3	25	0	0.00%	0.00%	13.32%
15	1	269	2	0.74%	0.20%	2.67%
15	2	563	2	0.36%	0.10%	1.29%
15	3	681	2	0.29%	0.08%	1.06%
15	4	224	0	0.00%	0.00%	1.69%
15	5	99	1	1.01%	0.18%	5.50%
15	6	226	9	3.98%	2.11%	7.39%
15	7	2009	34	1.69%	1.21%	2.36%
15	8	411	4	0.97%	0.38%	2.48%
17	1	197	10	5.08%	2.78%	9.09%
17	2	2514	39	1.55%	1.14%	2.11%
17	3	201	0	0.00%	0.00%	1.88%
17	4	191	0	0.00%	0.00%	1.97%
18	1	2300	3	0.13%	0.04%	0.38%
18	2	596	3	0.50%	0.17%	1.47%
Total		**10900**	**111**	**1.02%**	**0.85%**	**1.22%**

There were 70 lines of pastoral sheep from New South Wales exported on 8 separate voyages. Average mortality for pastoral sheep from NSW was 2.83% (2.67%, 3.00%). There were 20 lines in which mortality exceeded 2%, which is the reportable mortality level set out in the Australian standards for the export of livestock.

The mortality for the NSW pastoral sheep varied by district. At the commencement of the project, NSW districts were referred to as Rural Lands Protection Board (RLPB) Districts and the property identification code of each line of sheep received was used to trace the sheep to their district of origin. Since then, the RLPB districts have been amalgamated into Livestock Health and Pest Authorities (LHPA). Figure 8.4 shows the current LHPA zoning and the former RLPB districts. Sheep sourced from the far west of NSW had the highest mortality with sheep from the former RLPB districts of Milparinka, Broken Hill, Wanaaring and Wilcannia having the highest mortality.

Table 8-7 Summary statistics for pastoral sheep from NSW including voyage code, line code, number accepted, mortality count and mortality % with 95% confidence intervals

					95% CI	
Voyage	Line	n	Mortality Count	Mortality %	Lower	Upper
1	1	802	0	0.00%	0.00%	0.48%
1	2	745	2	0.27%	0.07%	0.97%
1	3	388	0	0.00%	0.00%	0.98%
1	4	951	2	0.21%	0.06%	0.76%
15	1	258	0	0.00%	0.00%	1.47%
15	2	291	1	0.34%	0.06%	1.92%
17	1	591	21	3.55%	2.34%	5.37%
17	2	1230	30	2.44%	1.71%	3.46%
17	3	278	3	1.08%	0.37%	3.12%
21	1	616	193	31.33%	27.79%	35.10%
21	2	2387	143	5.99%	5.11%	7.02%
21	3	798	10	1.25%	0.68%	2.29%
21	4	692	42	6.07%	4.52%	8.10%
21	5	536	36	6.72%	4.89%	9.16%
21	6	404	15	3.71%	2.26%	6.03%
21	7	279	11	3.94%	2.22%	6.92%
21	8	7	0	0.00%	0.00%	35.43%
21	9	464	219	47.20%	42.70%	51.74%
21	10	622	2	0.32%	0.09%	1.16%
21	11	969	15	1.55%	0.94%	2.54%
21	12	961	56	5.83%	4.51%	7.49%
21	13	87	1	1.15%	0.20%	6.23%
21	14	597	4	0.67%	0.26%	1.71%
21	15	1353	0	0.00%	0.00%	0.28%
21	16	605	2	0.33%	0.09%	1.20%
21	17	249	0	0.00%	0.00%	1.52%
21	18	162	0	0.00%	0.00%	2.32%
22	1	573	9	1.57%	0.83%	2.96%
22	2	767	24	3.13%	2.11%	4.61%
22	3	784	1	0.13%	0.02%	0.72%
22	4	399	4	1.00%	0.39%	2.55%
22	5	432	1	0.23%	0.04%	1.30%
22	6	454	0	0.00%	0.00%	0.84%
22	7	1205	1	0.08%	0.01%	0.47%
22	8	316	1	0.32%	0.06%	1.77%
22	9	352	0	0.00%	0.00%	1.08%
22	10	505	5	0.99%	0.42%	2.30%
22	11	247	1	0.40%	0.07%	2.26%
22	12	495	68	13.74%	10.98%	17.05%
22	13	582	4	0.69%	0.27%	1.75%
22	14	319	0	0.00%	0.00%	1.19%
22	15	713	8	1.12%	0.57%	2.20%
22	16	130	4	3.08%	1.20%	7.64%
22	17	106	0	0.00%	0.00%	3.50%
22	18	57	1	1.75%	0.31%	9.29%
22	19	28	3	10.71%	3.71%	27.20%

Voyage	Line	n	Mortality Count	Mortality %	95% CI Lower	95% CI Upper
22	20	620	0	0.00%	0.00%	0.62%
22	21	294	6	2.04%	0.94%	4.38%
22	22	420	1	0.24%	0.04%	1.34%
22	23	615	15	2.44%	1.48%	3.98%
22	24	154	0	0.00%	0.00%	2.43%
22	25	93	3	3.23%	1.10%	9.06%
22	26	1	0	0.00%	0.00%	79.35%
22	27	303	0	0.00%	0.00%	1.25%
22	28	302	0	0.00%	0.00%	1.26%
23	1	1327	7	0.53%	0.26%	1.08%
23	2	976	9	0.92%	0.49%	1.74%
23	3	607	3	0.49%	0.17%	1.44%
23	4	611	1	0.16%	0.03%	0.92%
38	1	596	2	0.34%	0.09%	1.22%
38	2	445	1	0.22%	0.04%	1.26%
38	3	368	1	0.27%	0.05%	1.52%
38	4	342	11	3.22%	1.81%	5.67%
38	5	557	2	0.36%	0.10%	1.30%
38	6	898	56	6.24%	4.83%	8.01%
39	1	41	2	4.88%	1.35%	16.14%
39	2	252	0	0.00%	0.00%	1.50%
39	3	350	0	0.00%	0.00%	1.09%
39	4	1630	2	0.12%	0.03%	0.45%
39	5	39	0	0.00%	0.00%	8.97%
Total		**37627**	**1065**	**2.83%**	**2.67%**	**3.00%**

Table 8.8 summarises the performance of the NSW pastoral sheep by district. There were four lines of sheep in the final dataset that were received as pastoral sheep for the purposes of the trial but their property identification code was not recorded. These 2,886 sheep were excluded from this table.

Table 8-8 Mortality for pastoral sheep from NSW by distinct of origin.

Former RLPB District	Number of lines	Sheep	Mortality Count	Mortality %	95% CI Lower	95% CI Upper
Balranald	5	1,826	9	0.49	0.26	0.93
Bourke	2	1,235	15	1.21	0.74	1.99
Broken Hill	17	6,236	328	5.26	4.73	5.84
Hillston	7	4,839	14	0.29	0.17	0.49
Milparinka	5	4,127	328	7.95	7.16	8.81
Wanaaring	8	4,693	152	3.24	2.77	3.78
Wentworth	2	605	0	0.00	0.00	0.63
Wilcannia	20	11,180	215	1.92	1.68	2.19
Total	**66**	**34,741**	**1061**	**3.05**	**2.88**	**3.24**

Figure 8-4 NSW Former Rural Lands Protection Board Districts (LHPA 2009)

8.2.4 Spatial analysis

Figure 8.5 shows the locations from which sheep were sourced. Figure 8.6 shows only properties that had mortality with locations colour coded to indicate mortality rate. Spatial autocorrelation was applied separately to each port of loading to look for evidence of mortality clustering by location. There was no indication of clustering by location.

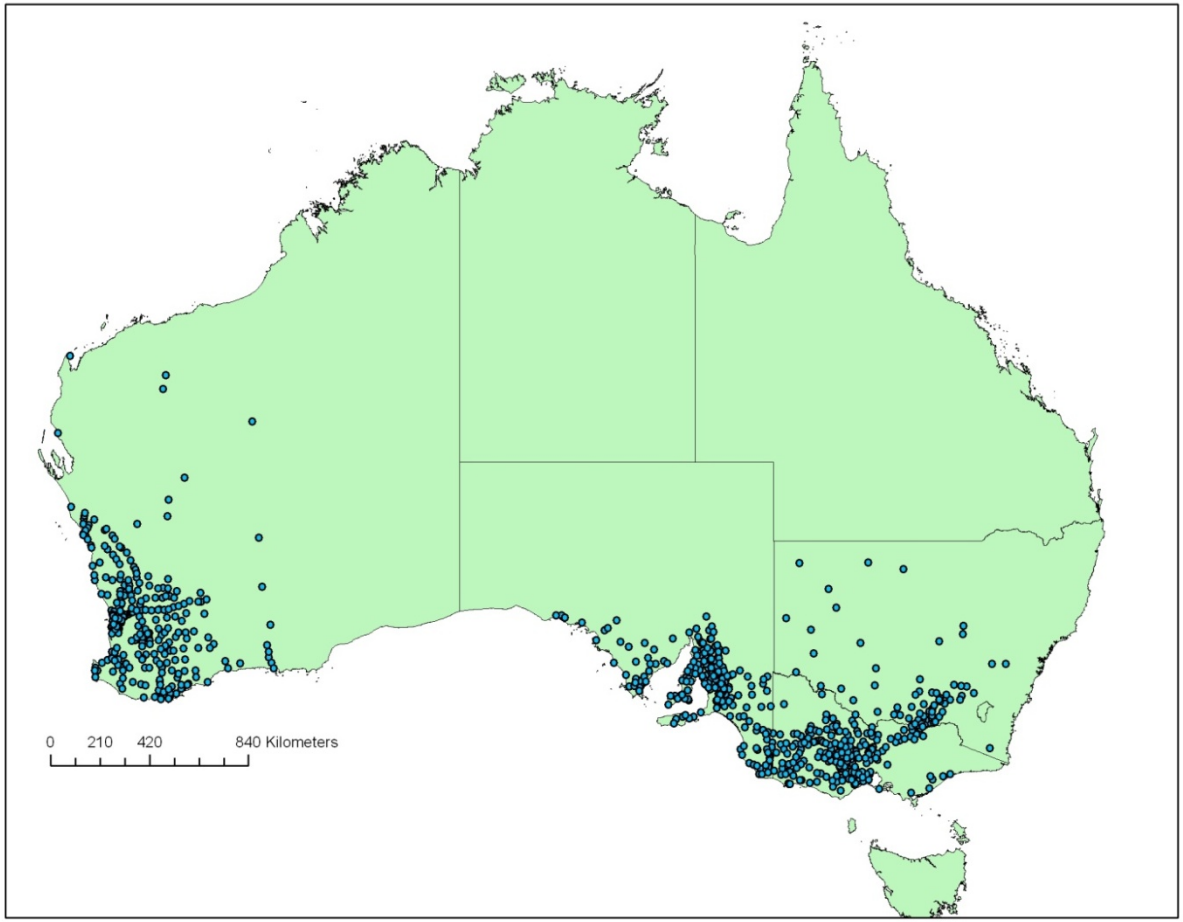


Figure 8-5 Locations of properties from which sheep were sourced

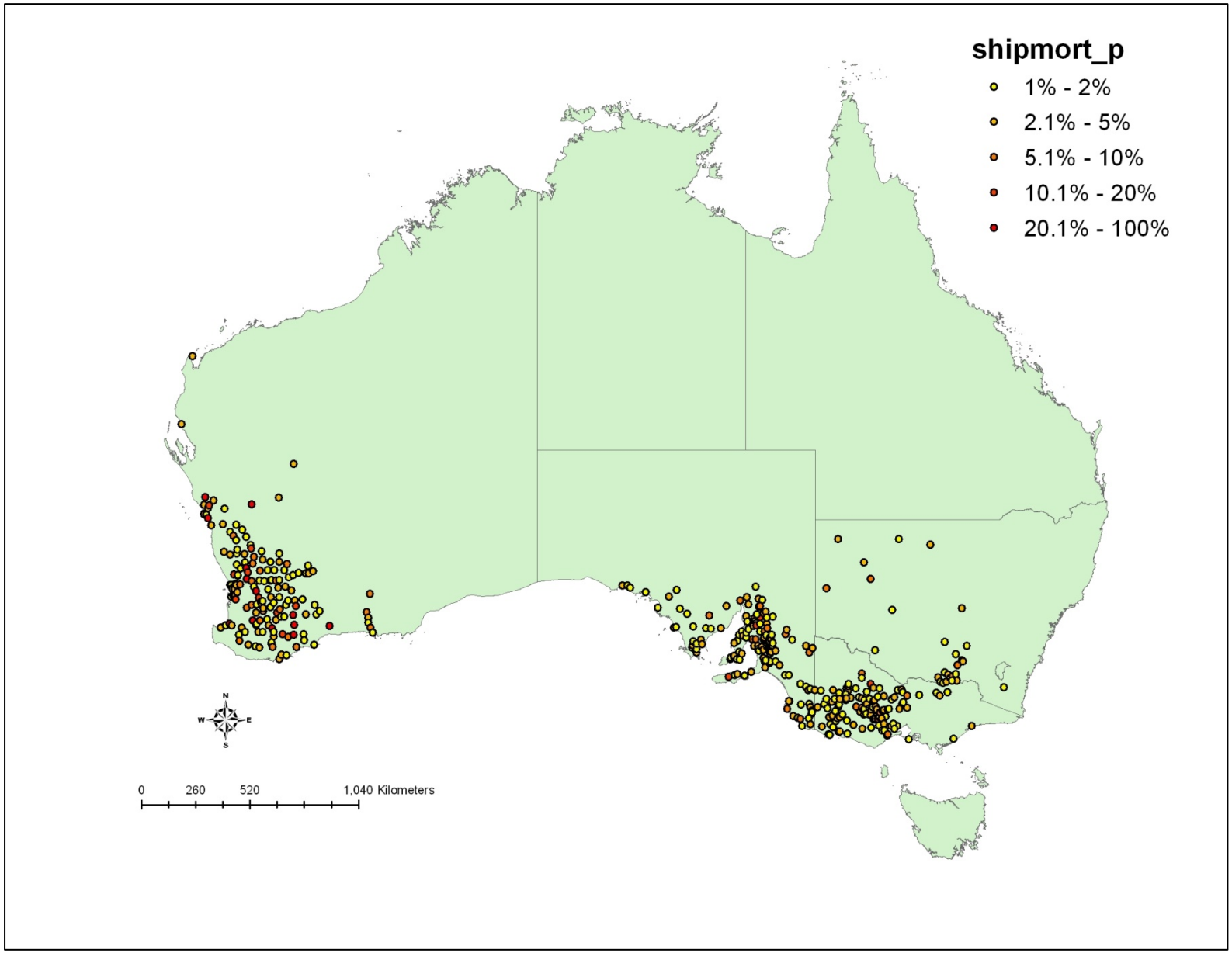


Figure 8-6 Locations of properties that experienced mortality colour coded by mortality percentage

8.2.5 Multivariate analysis

Univariate analysis found significant variation in the relative risk of mortality by voyage, month, season, year, port of loading, assembly depot, state, class of sheep, age of sheep, distance travelled, ship, duration of assembly period and property of origin rainfall and average maximum daily temperature.

Correlation of these variables revealed colinearity between month and season, voyage and year, port and state, port and assembly depot and several of the recorded weather variables. Season, port, year, and two weather variables were included in the initial multivariate model, season was the only one of these variables that remained in the final multivariate model. The scale of continuous variables was checked and distance was found to have a non-linear relationship with the outcome and so was categorised and included in the analysis. The backwards stepwise approach was used to remove the least significant variables from the model one at a time. No significant interaction terms were identified. No problems were identified with overall model fit or residuals.

Table 8.9 results of the final multivariable negative binomial regression for mortality during the voyage. In this model vendor was added as a cluster variable to account for variation between vendors.

Table 8-9 Results of final multivariable negative binomial regression model for voyage mortlaity. (n = number of sheep, RR = relative risk, CI = confidence interval)

					95% CI		
Variable	**Category**	**N**	**Deaths**	**RR**	**Lower**	**Upper**	**p-value**
Year	**2008**	**69,049**	**221**	*Reference*			
	2005	**160,699**	**1,306**	2.08	1.32	3.28	0.002
	2006	**633,667**	**4,287**	1.74	1.20	2.52	0.003
	2007	**786,565**	**5,746**	1.59	1.08	2.35	0.020
Port of Loading	**Portland**	**533,408**	**3,217**	*Reference*			
	Adelaide	**471,925**	**4,355**	1.34	1.11	1.63	0.003
	Fremantle	**644,647**	**3,988**	1.20	1.04	1.39	0.012
Distance	**<200**	**537,894**	**3,459**	*Reference*			
	200-299	**368,105**	**2,180**	0.87	0.76	0.99	0.040
	300-499	**357,207**	**2,306**	0.98	0.86	1.13	0.812
	500-799	**287,390**	**2,997**	1.08	0.92	1.27	0.320
	>800	**68,866**	**555**	0.94	0.69	1.27	0.678
Pastoral	**No**	**1,601,453**	**10,384**	*Reference*			
	NSW	**37,627**	**1,065**	3.29	1.87	5.76	<0.001
	SA	**10,900**	**111**	1.32	0.68	2.55	0.408
Age	**Old**	**1,094,860**	**9,323**	*Reference*			
	Lamb	**119,055**	**683**	1.22	0.77	1.93	0.403
	Young	**436,065**	**1,554**	0.53	0.47	0.59	<0.001
Sex	**Wether**	**1,447,794**	**10,077**	*Reference*			
	Unknown	**110,713**	**483**	0.48	0.30	0.79	0.004
	Ram	**51,847**	**608**	1.19	0.97	1.47	0.095
	Ewe	**39,626**	**392**	1.13	0.85	1.51	0.400
QLD	**No**	**1,630,901**	**11,170**	*Reference*			
	Yes	**19,079**	**390**	2.87	1.39	5.91	0.004
Ship	**4**	**18,408**	**20**	*Reference*			
					95%		

Variable	Category	N	Deaths	RR	CI Lower	CI Upper	p-value
	1	**321,753**	**2,041**	3.59	1.43	8.97	0.006
	2	**492,174**	**3,317**	4.16	1.68	10.35	0.002
	3	**572,352**	**3,489**	3.49	1.41	8.61	0.007
	5	**114,459**	**803**	3.07	1.21	7.79	0.018
	6	**130,834**	**1,890**	4.57	1.79	11.68	0.001
Season	**Autumn**	**345282**	**1758**	*Reference*			
	Summer	**127312**	**733**	1.28	1.01	1.62	0.038
	Winter	**626880**	**5027**	1.36	1.12	1.64	0.002
	Spring	**550506**	**4042**	1.35	1.11	1.63	0.002
Breed	**Merino**	**1601854**	**11168**	*Reference*			
	Damara	**22563**	**316**	0.90	0.50	1.63	0.728
	Dorper	**5578**	**16**	0.42	0.09	2.00	0.277
	XB	**19985**	**60**	0.55	0.38	0.79	0.001
Duration (days)	**3 - 6**	**476077**	**3374**	*Reference*			
	7 - 8	**698551**	**5170**	0.82	0.71	0.94	0.004
	9 - 32	**475352**	**3016**	0.93	0.77	1.13	0.458
Property Temp				0.982	0.96	1.00	0.074
Property Rain				1.002	1.00	1.00	0.077

8.3 Discussion

8.3.1 Traceability of mortality

There are a number of reasons for the differences in traceability across ports. The development of the information management system began in 2005/2006 with the assembly depots in Portland. The assembly depot operators and buyers were very helpful providing input in regards to needs and supported the concept at the onset of the project. At these assembly depots it is also standard policy for the operators at the unloading ramps to check the identity of the sheep and to report the identity on the docket that is used for entering data at the weigh bridge.

The volume of sheep exported from South Australia in 2006 was small so the information management system was not installed in South Australia until later in the year. In South Australia one of the assembly depots routinely verifies the identity of sheep and the other relies on the identity recorded by the buyers.

West Australia represents a more complex system than the east in that the number of shipments is greater and it is common for sheep consignments to “carry over” from one voyage to the next. During times of sheep shortage, sheep may be accumulated over a longer period of time for more

than one shipment. Consequently traceability of sheep performance in West Australia really requires consistent recording of vendor details across all voyages. It is not always possible to know what proportion of a consignment is loaded on which ship. It is subsequently more difficult to follow and compare outcomes of different lines of sheep with this system. Another complexity seen more commonly in West Australia is the chartering of ships with multiple exporters on the same ship. This requires all exporters to provide consignment data to account for the observed mortalities. If this data is not available you are not able to determine if the untraceable mortality is attributable to unidentified “carry over” sheep or sheep from another vendor. The load plan is helpful in this regard, however on some ships dead sheep are placed on lifts and it can be difficult to determine where they came from.

Katanning and Midland sale sheep represent a significant number of export sheep in West Australia. The identity of these sheep are not routinely electronically recorded in the receival files. The identity of the sheep could be traced by the National Vendor Declarations but is not in an easily retrievable form.

The West Australian tags also present some difficulties in regards to the accuracy of data recording. In contrast to the NLIS ear tag numbers that include eight characters the West Australian tags contain three characters. One of the three characters on West Australian tags can be orientated on its side (Lazy) to increase the number of possible unique property identities. The lazy characters are often misread resulting in the creation of a fictitious identity. As the tag only contains three characters it is often impossible to deduce the origin of the tag from the assembly receival records when there is an error in one character.

8.3.2 Line

The finding that 74% of all mortality was traced to 18% of lines was in agreement with previous studies that found mortality rates in sheep exported live by sea vary widely between farm groups, with certain lines of sheep suffering significantly higher mortality than others. Norris et al. (1989) found that 54% of all deaths during export occurred in only 25% of lines. In addition to this, Higgs et al. (1999) found that mortality rates in different farm groups ranged from 0 to 28.2%, with half of all sheep that died being traced to 14.2% of consignments (Norris, Richards et al. 1989; Higgs, Norris et al. 1999).

8.3.2.1 Pastoral and Queensland sheep

Pastoral sheep from NSW had a significantly higher risk of mortality, being 3.29 times more likely to die than those not from pastoral areas. Sheep from Queensland sourced from southern vendors had a relative risk of mortality 2.87 times higher than sheep not from Queensland. Higher mortality lines were most notable in the latter half of 2007. A large proportion of the mortality for Queensland and pastoral sheep was attributable to a small proportion of vendors.

It was notable that pastoral sheep sourced from NSW had an increased risk of mortality whereas pastoral sheep sourced from South Australia did not. This suggests the mortality is related to a regional phenomena rather than pastoral status per se. In regards to finding answers for producers from the western division of NSW further research is required. Project team members travelled with consignments of these sheep and investigated high mortality consignments. In each case the consignments experienced outbreaks of salmonellosis. Interestingly these sheep often presented with full rumens particularly at the start of the outbreaks suggesting that inappetence was not necessarily the predisposing factor. It is also notable that in the Human population of Broken Hill heavy lead toxicosis is recognized as a human health problem that is associated with the soil type

and mines (Lyle et al. 2006). Chronic exposure to lead is known to be immunosuppressive (Fernandez-Cabezudo et al. 2003). It would be interesting to investigate the heavy metal status of sheep from the properties that experienced high mortality to determine if a similar problem is present in the sheep population of this region.

8.3.3 Year and port

For the voyages studied, there was significant variation in mortality by year with sheep exported in 2008 having a lower relative risk of mortality than sheep exported in all other years. This finding is consistent with the National Livestock Export Industry Shipboard Performance Report 2008 which is an annual report on the performance of all livestock exported by sea. The report showed that the mortality rate for sheep exported by sea in 2008 was 0.84 % (Norman and Norris 2009). In this study there was also significant variation in mortality by port of loading with sheep exported from Portland having the lowest risk of mortality. This was also noted in the report by Norman and Norris but by comparison, sheep exported from Portland only had a lower mortality rate in 2008, in all other years covered by the report Fremantle sheep had the lowest mortality rate. This difference is likely due to the fact that the Norman and Norris report covers a larger number of voyages and does not use multivariate analysis.

8.3.4 Distance

Distance travelled from the property of origin to the assembly depot was included in the analysis. This variable remained in the model although there was only one significant comparison between categories with sheep travelling 200-299 km to reach the assembly depot having a lower risk of mortality than those travelling less than 200 km. While one significant comparison was identified, distance travelled to reach the assembly depot did not appear to have a consistent effect on the risk of mortality.

8.3.5 Age

The relative risk of mortality in young wethers was 53% of that for old wethers, there was also no statistically significant difference between old wethers and lambs. “Young” weathers have six or less adult incisors and “old” wethers eight adult incisors. The observation in this study that mortality varies by class of sheep is in agreement with previous studies that found different classes of sheep vary in their ability to adapt to sea transport. Higgs et al. (1991) analysed 50 shipments and found lower mortality rates in wether hoggets (0.52%) than adult wethers (1.63%) (Higgs, Norris et al. 1991).

8.3.6 Sex

Compared to wethers the risk of mortality was higher in rams (1.19 times higher), although this difference was not highly significant. There was no significant difference between wethers and ewes. Sheep in the unknown sex category are those for which sex was not specified on receival, for example ‘merino lambs’. This finding is also consistent with the National Livestock Export Industry Shipboard Performance Report 2008 which reported that the mortality rates for rams of all ages were higher than all other classes of sheep.

8.3.7 Ship

Mortality also varied significantly between ships with ship 4 having a significantly lower risk of mortality than all other ships. The relative mortality risk for all other ships ranged from 3.07 to 4.57 times higher than that for ship 4. There is significant variation between ships in design and management of sheep, however, this finding should be interpreted with caution as there was only one small consignment of sheep exported on ship 4 included in the analysis.

8.3.8 Season

Mortality was significantly higher in winter and spring than in autumn. This effect is likely due to a combination of factors including seasonal variations in sheep appetite drive and metabolism and the extremes of heat and humidity experienced in the Persian Gulf during northern hemisphere summer. Previous studies demonstrated that time of year has a significant effect on sheep appetite drive and metabolism and subsequently performance and mortality on ship (Higgs, Norris et al. 1991) with mortality rates being higher in the second half of the year. In addition to this, Kelly (1996) observed increased mortality during periods of high temperature and humidity, as are encountered during Middle Eastern summer from July to September. This increase in mortality was not due to heat stress deaths but to an increase in other causes of mortality (primarily inanition) (Kelly 1996).

8.3.9 Other factors

The remaining factors in the multivariate model were initially excluded from the model but were later added back as they had a confounding effect on other variables. There was one significant comparison between breeds of sheep with cross bred sheep having a lower mortality risk than merino sheep. The duration of the pre-export assembly period was also added back and sheep assembled for 7 to 8 days had a lower risk of mortality than those assembled for 3 to 6 days. The average maximum daily temperature and total rainfall at the property of origin for the 30 days prior to delivery to the assembly depot did not have a significant effect on mortality risk.

8.3.10 Summary of important findings

- There was significant variation in mortality between voyages
- 62.7% of lines had no recorded sheep mortalities
- 74% of all mortality was traced to 18% of lines
- The risk of mortality was significantly higher for sheep from the pastoral zone of NSW and from Queensland
- Distance travelled to reach the assembly depot did not have a marked effect on the risk of mortality
- Mortality risk is lower for young wethers than for old wethers
- There was a increased risk of mortality in rams compared to wethers, although the difference was not highly significant
- There was significant variation in mortality risk between ships
- The risk of mortality was significantly higher in winter and spring than in autumn

9 Voyage mortality - postmortems

9.1 Materials and methods

9.1.1 Postmortem examinations

Five voyages were accompanied by a member of the research team. For these voyages, postmortem examinations were carried out on an opportunistic sample of sheep that died. Sheep that die on ship are pulled out of the pens and their details recorded each morning. This represents the opportunity to conduct postmortems. The time available to conduct postmortems is dependent on the ambient temperature. As temperature and humidity rise the time available to conduct postmortems is limited by the rapid onset of autolysis (decomposition). Postmortems were also not conducted in port due to the problem that this created with carcass disposal. Despite the fact that postmortems were only conducted on a portion of the sheep that died we do not anticipate that this opportunistic sampling biased the findings. Ship board veterinarians recorded postmortem information on a further 14 voyages. A summary of postmortems performed is presented in table 9.1.

Table 9-1 Mortality count, postmortem count and percentage of postmortems performed by voyage

Voyage	Mortality Count	Postmortems	Voyage	Mortality Count	Postmortems
1	1160	367	**14**	491	329
		31.60%			67.0%
3	522	156	**15**	400	210
		29.9%			52.5%
4	611	183	**16**	372	154
		30.0%			41.4%
5	518	91	**17**	1611	542
		17.6%			33.6%
8	572	310	**18**	358	278
		54.2%			77.7%
9	865	123	**19**	719	444
		14.2%			61.8%
10	690	411	**20**	1127	514
		59.6%			45.6%
11	883	605	**21**	1494	176
		68.5%			11.8%
12	655	165	**22**	964	310
		25.2%			32.2%
13	462	289			
		62.6%			

Postmortem investigations included a general examination of the carcass for evidence of trauma or subcutaneous oedema as may be observed with urethral rupture secondary to urinary calculi. The sheep was laid right side down and the left front and hind legs reflected along with the skin on the neck. Following opening of the abdomen the rib cage was reflected to expose the heart and lungs. The rumen, abomasum, and omasum were reflected caudally and stretched out behind the sheep prior to examination of the contents. Following examination of the lungs, heart, liver, spleen, kidneys, bladder, rumen, omasum, abomasum, duodenum, jejunum, ileum, caecum, and spiral colon digital images were taken of gross lesions, rumen fill and rumen contents. Rumen pH was evaluated for the first 3 days of the voyage to check for ruminal acidosis and swabs of gut contents and liver or spleen were collected each day from a sample of cases that presented with gross pathology compatible with salmonellosis. The post mortem examinations conducted did not include examination of the brain or spinal cord. Tissue samples were collected from a subset of cases and preserved in formalin. The tentative diagnoses recorded during the voyage were based on gross pathology and in the case of sheep euthanased *in extremis* clinical signs observed prior to death. Criteria for mortality classification are shown in appendix 1.

9.1.2 Salmonellosis treatment trial

An antibiotic treatment trial was carried out during voyage 22. Previously we had observed variation in shipboard disease management. In regards to therapeutic management of sick sheep on ship different approaches were employed by different shipboard veterinarians, these included no treatment, feeding chaff, injecting individual sheep with tetracycline, and pen medication with tetracycline. In the absence of controlled clinical trials it is difficult to advocate one approach over another. Overt clinical salmonellosis and high mortality was observed in a group of rams loaded in Adelaide. In an attempt to manage this problem and to determine the relative efficacy of alternate therapeutic approaches a treatment trial was conducted. The rams were located in 3 adjacent pens, 2 pens contained 230 head and one 180 head. One pen was managed by injecting sick sheep with oxytetracycline intramuscularly with a dose of 10 mg/kg when they were observed to be ill. The second pen was medicated with apramycin administered in the water at a dose of 10 mg/kg daily. The third group was medicated orally with tetracycline administered in the water at a dose of 20 mg/kg daily. All groups were treated for 5 days. All sheep were also provided access to chaff which they ate in preference to the pellets. At post mortem it was observed that many of the sheep had been eating the pellets prior to the outbreak of salmonellosis in the group.

9.2 Results

9.2.1 Postmortem examinations

The post-mortem examination results presented in figure 9.1 indicate the proportion of diagnosed voyage mortalities attributed to each of the nine most frequent diagnoses. Unexamined mortalities and mortalities where no diagnosis was made are not included.

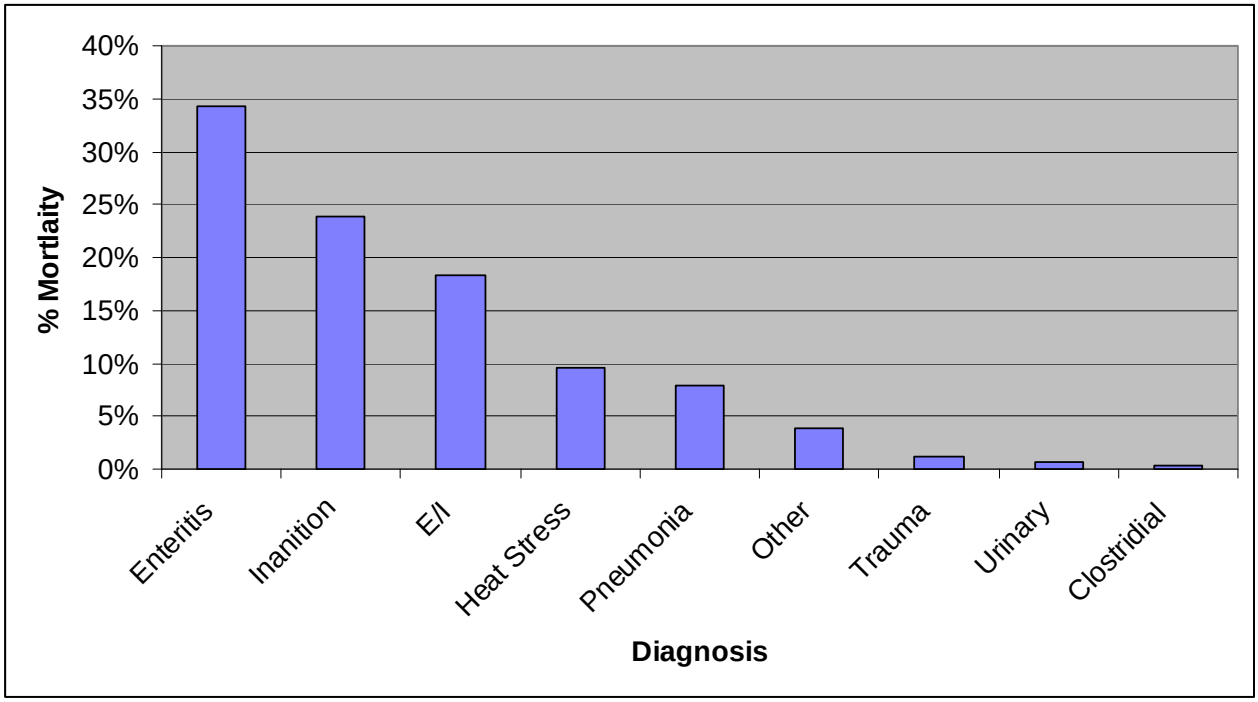


Figure 9-1 Percentage of voyage mortality attributable to each of nine diagnostic categories across all 19 voyages on which postmortem examinations were performed

Enteritis and inanition continue to contribute significantly to mortality in sheep exported live by sea, accounting for over 76% of diagnosed mortality. In earlier studies (Richards, Norris et al. 1989) inanition alone was shown to cause 43.4% of deaths and enteritis was shown to cause 20.2% of mortality. In this study, enteritis was found to be the most common cause of mortality (34.4%), followed by inanition (23.9%) and enteritis/inanition (18.2%).

Figure 9.2 shows the breakdown of post-mortem results by voyage for the six most common diagnostic categories. The height of the bar indicates the overall mortality rate as deaths per 10,000 sheep. The coloured sections indicate the mortality count for each diagnosis.

It is assumed that the results for diagnosed mortality are reflective of total mortality, i.e. if 50% of diagnosed mortality was caused by enteritis, then 50% of total voyage mortality is assumed to be caused by enteritis. Figure 9.2 shows that enteritis, inanition and a combination of the two were the most common diagnoses across all voyages. However, the proportion of mortality attributable to enteritis or inanition varies between voyages.

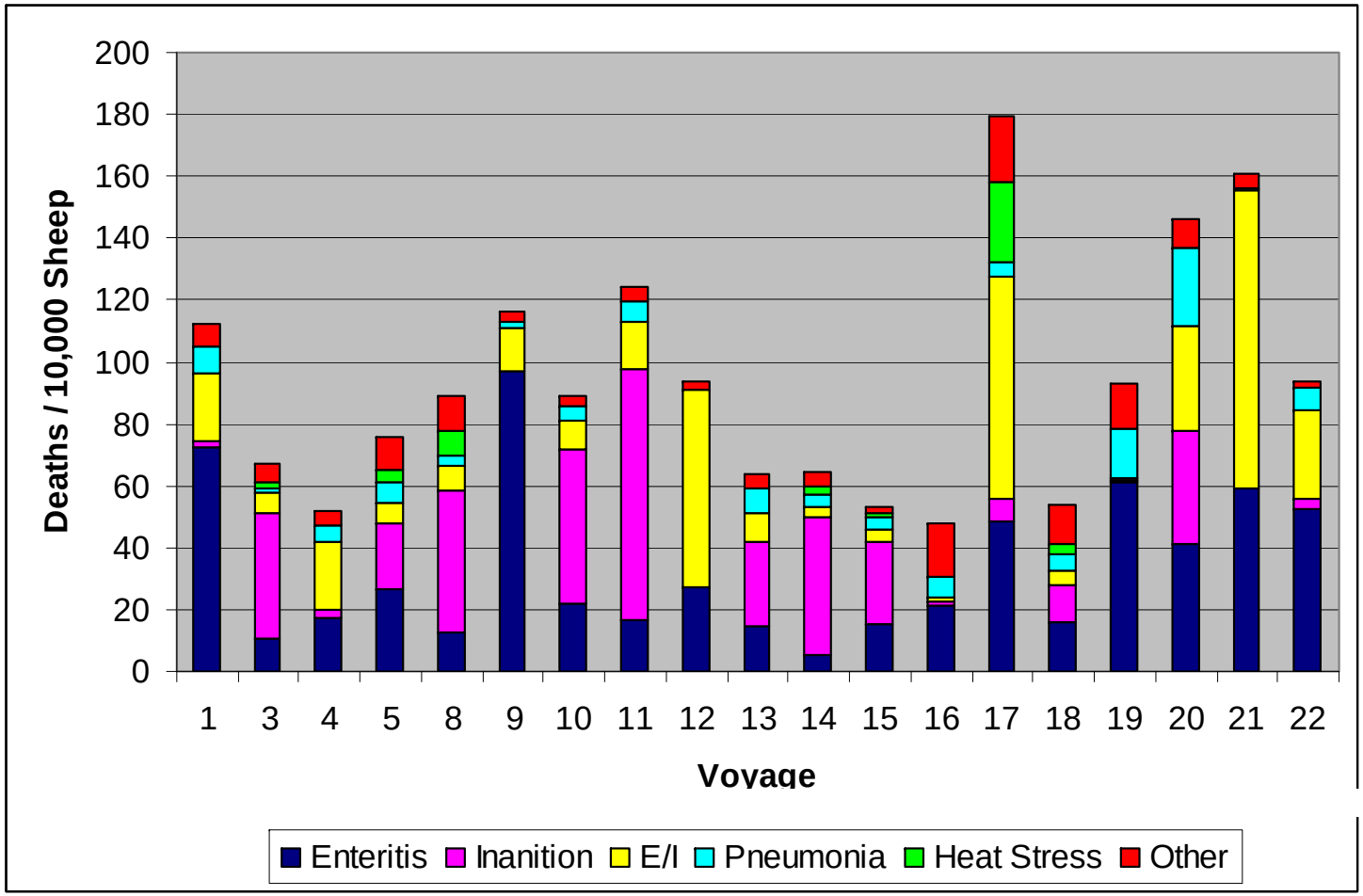


Figure 9-2 Mortality per 10,000 sheep for 19 voyages. The height of each bar represents the number of mortalities/10,000 sheep and the coloured sections represent the proportion of mortality attributable to each of the 6 mortality categories

Post-mortem result by day of voyage is illustrated in figures 9.3 and 9.4.

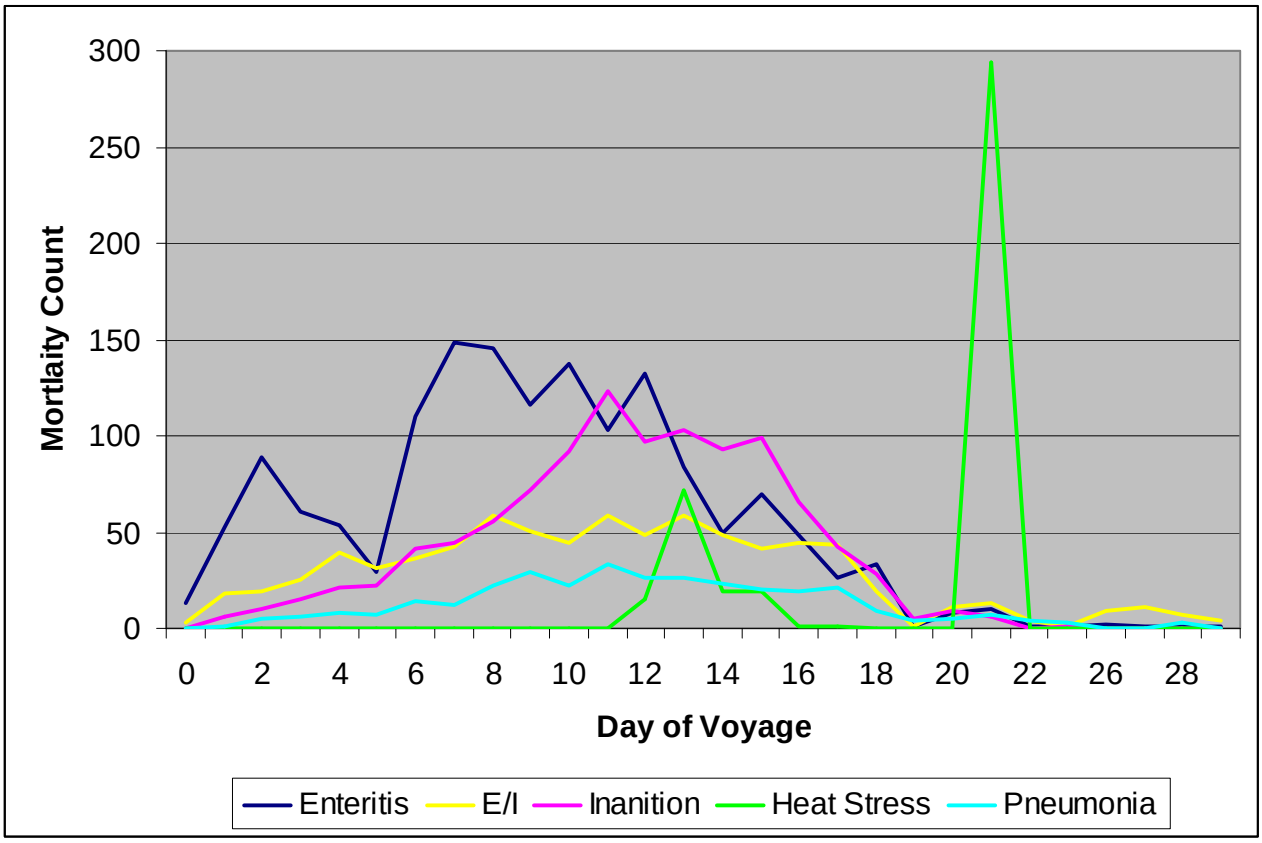


Figure 9-3 Mortality count for each of four major diagnoses by day of voyage across all 19 voyages

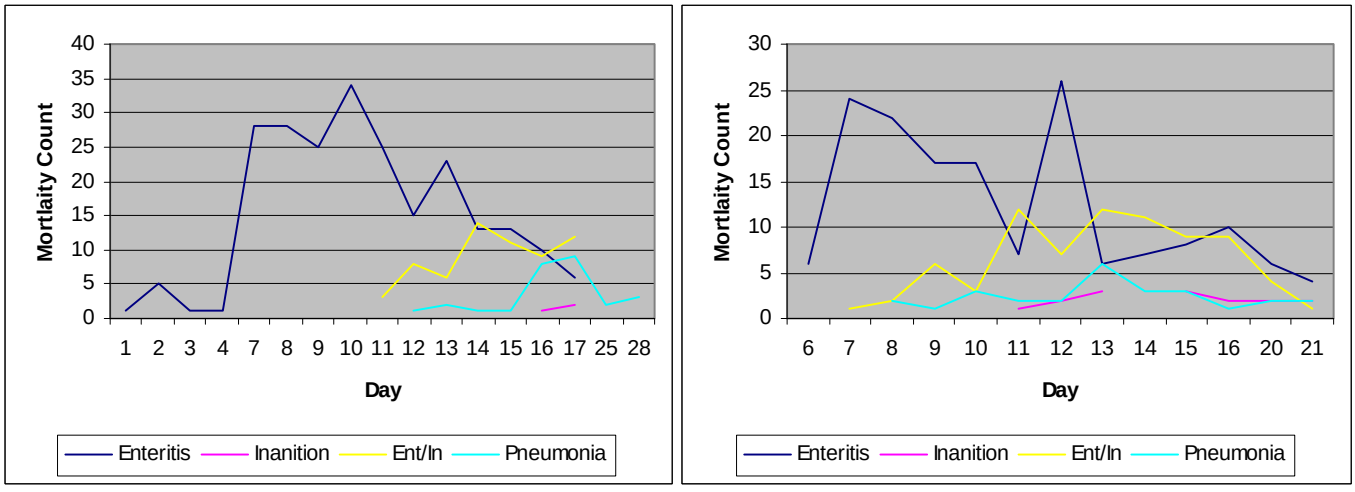


Figure 9-4 Voyage 1 (left) and 22 (right) mortality count for each of four major diagnoses by day of voyage

The figures show that enteritis is the dominant cause of mortality early in the voyage with inanition and pneumonia contributing more significantly to mortality towards the end of the voyage. All these figures show two peaks for enteritis. The first peak represents enteritis mortality among sheep loaded at the eastern ports; the second peak represents enteritis mortality among sheep loaded in West Australia.

Mortality tends to peak during times of high temperature and humidity. The heat stress peaks in figure 9.3 represent heat stress events discussed above that occurred on voyages 8 and 17. In addition to this, mortality due to other causes also peaks at these times. Early in the voyage most of the sheep diagnosed with enteritis had relatively full rumens. Dehydration is seen as the temperature increased with proximity to the equator and later in the gulf. A variable that appears to contribute to the demise of compromised sheep during hot weather is a reduced drive to gain access to water.

Figure 9.5 is a survival curve for voyage 1 from a Cox proportional hazards regression. Proportion survival over time is shown by port. Mortality is presented by day of voyage, with the first mortality count occurring on day 1 of the voyage. The highest incidence of mortality was observed in Fremantle loaded sheep between voyage days 7 to 14 (as indicated by larger steps in the graph early in the voyage). The Fremantle sheep were loaded on day 5 so this period reflected the first 9 days 2 to 9 for these sheep. Early mortality in Fremantle sheep was attributed to acute salmonellosis. Inanition accounted for a higher proportion of the mortality during the later portion of the voyage. Interestingly the incidence of mortality in Fremantle sheep continued to exceed mortality in Portland loaded sheep following the resolution of acute salmonellosis suggesting that those animals surviving the acute phase of the disease remained compromised for the remainder of the voyage.

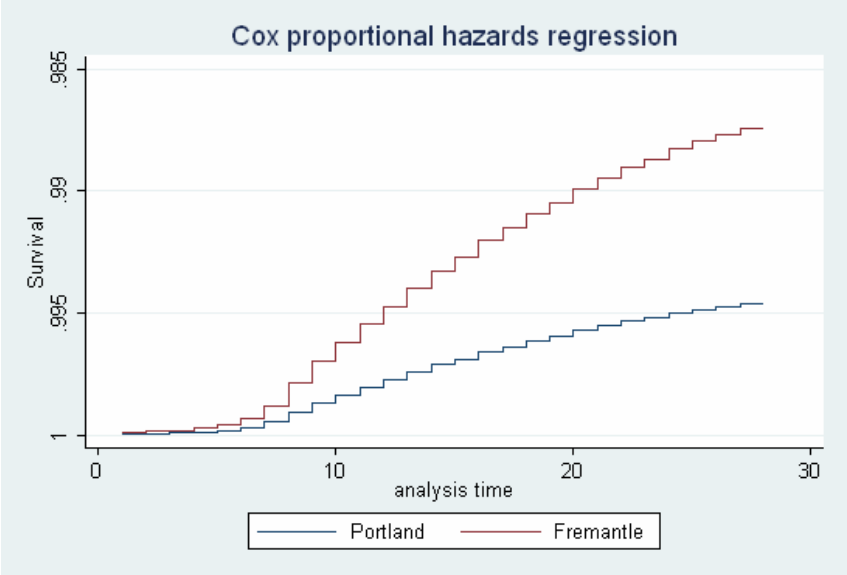
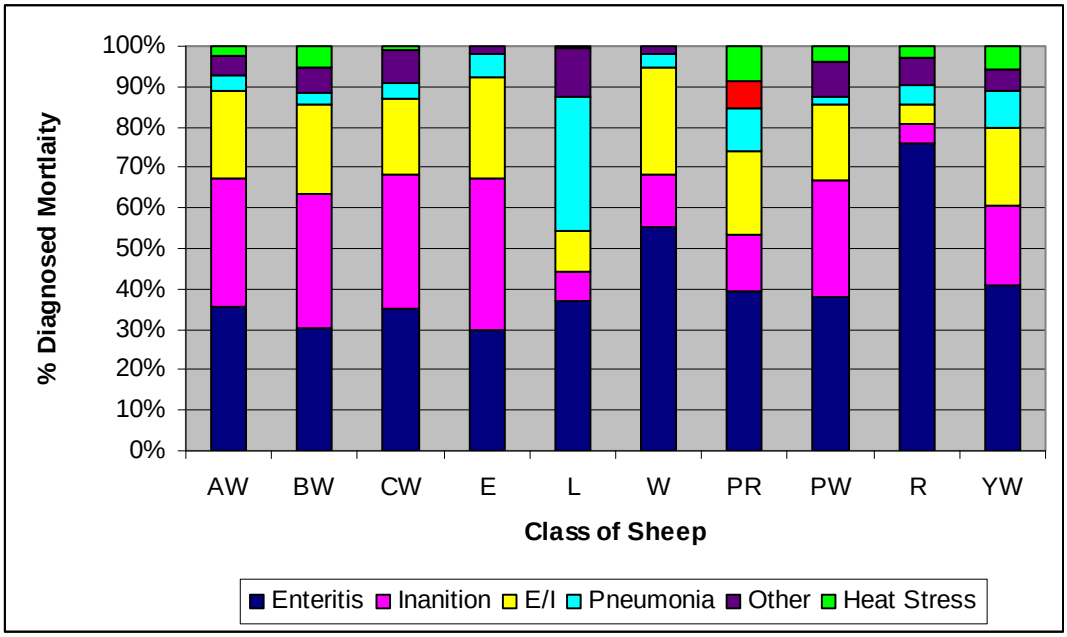


Figure 9-5 Survival curve by port of loading for voyage 1

Figure 9.6 shows post-mortem diagnosis results by class. Included are post-mortems where a diagnosis was made.

Key - AW/BW/CW = A/B/C class weather, E = Ewe, L = Lamb, W = Wether (no class), PR = Pastoral Ram, PW = Pastoral Wether, R = Ram, YW = Young Wether

Figure 9-6 Post-mortem diagnosis by class. For each class the relative contribution (%) to diagnosed mortality of each of the major 6 diagnoses are shown



The most notable feature regarding the distribution of mortality by sheep class is the higher proportion of mortality in lambs attributed to pneumonia. Pneumonia was a relatively common cause of mortality in lambs and an infrequent cause of mortality in older sheep.

9.2.2 Salmonella culture

For the voyages accompanied by a member of the research team, tissue swabs were collected from sheep that succumbed to enteritis. These samples were cultured to determine if salmonella bacteria were present. Table 9.2 summarises samples collected and the proportion of salmonella positive cultures.

Table 9-2 Mortality count, postmortems performed, samples collected and % positive for salmonella by voyage

Year	Month	Voyage	Mortality Count	Post-mortem	Samples	% positive
2005	September	1	1160	367 31.6%	139	66.9%
2006	April	4	611	183 30.0%	89	31.5%
2006	August	9	865	123 14.2%	60	46.7%
2007	June	17	1334	267 20.0%	144	0.0%*
2007	September	22	964	310 32.2%	205	62.4%

* Note no salmonella were isolated from these samples due to a thermostat failure in the incubator that was used to incubate the samples causing them to be destroyed.

Tissue (liver or spleen), mesenteric lymph node and gut content salmonella cultures were performed to identify the presence of salmonella at necropsy and to determine the relationship between the salmonella serovars associated with mortality and those isolated from the assembly depots. Swabs collected at necropsy were placed in transport media and retrieved from ships when they returned to Australia. While this was not an ideal culture technique it did provide a means of collecting culture samples in a logistically difficult situation. Salmonella were recovered from sheep tissues during all voyages sampled except voyage 17. Due to a thermostat failure, voyage 17 samples were incubated at an excessively high temperature and no bacterial growth was obtained. Table 9.3 details the salmonella serotypes isolated from ship postmortems. While it is possible to isolate salmonella from the mesenteric lymph nodes and gut contents of sheep that have not died of salmonellosis the gross pathology and concurrent isolation of salmonella from a diversity of sites provides a strong causal association.

Table 9-3 Salmonella serotypes isolated form voyage postmortem samples with number isolated by voyage

Voyage	Salmonella Serotype	Phage Type	Number Isolated
1	*Salmonella typhimurium*	135	36
	Salmonella bovismorbificans	24	35
	Salmonella infantis	-	4
	Salmonella typhimurium	RDNC	4
	Salmonella bovismorbificans	13	3

Voyage	Salmonella Serotype	Phage Type	Number Isolated
	Salmonella Havana	-	3
	Salmonella bovismorbificans	32	2
	Salmonella bredeney	-	2
	Salmonella typhimurium	30	2
	Salmonella typhimurium	195	1
	Salmonella typhimurium	105 var	1
4	*Salmonella typhimurium*	135	11
	Salmonella bovismorbificans	24	6
	Salmonella typhimurium	9	4
	Salmonella typhimurium	12	2
	Salmonella typhimurium	64	2
	Salmonella typhimurium	RDNC	2
	Salmonella typhimurium	193	1
9	*Salmonella bovismorbificans*	24	17
	Salmonella typhimurium	9	6
	Salmonella infantis	-	5
22	*Salmonella typhimurium*	135	26
	Salmonella bovismorbificans	24	23
	Salmonella anatum	-	14
	Salmonella chester	-	9
	Salmonella typhimurium	108	6
	Salmonella typhimurium	182	6
	Salmonella typhimurium	12a	6
	Salmonella ohio	-	5
	Salmonella tennessee	-	5
	Salmonella havana	-	4
	Salmonella newport	-	4
	Salmonella typhimurium	12	4
	Salmonella typhimurium	9	3
	Salmonella bovismorbificans	13	2
	Salmonella infantis	-	2
	Salmonella kottbus	-	2
	Salmonella typhimurium	RDNC/AO43	2
	Salmonella adelaide	-	1
	Salmonella muenchen	-	1
	Salmonella onderstepoort	-	1
	Salmonella subsp 1 ser rough:r:1,5	-	1
	Salmonella typhimurium	30	1

9.2.3 Heat stress events

The majority (70%) of heat stress deaths occurred on voyage 17 (334/480), these were excluded from the data in figure 9.6 and are presented below in figure 9.7. All heat stress deaths occurred on a single day (day 21); figure 9.7 shows how the different classes of sheep were affected by the event.

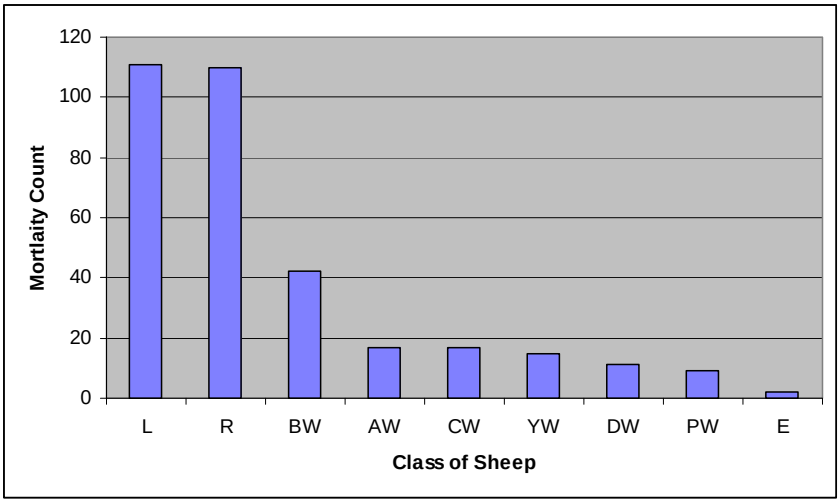


Figure 9-7 Voyage 17 heat stress mortality count by class.

Key - L = Lamb, R = Ram, BW/AW/CW/DW = B/A/C/D class weather
YW = Young Wether, PW = Pastoral Wether, E = Ewe

Lambs and rams were the most severely affected by the heat stress event. For the lambs this was determined to be a spatial effect as 101 of the 105 lambs that died were on deck 11. Mortality for lambs on deck 11 was significantly higher (1.47%) than that for lambs on other decks (0.37%). Deck 11 is the top deck of the sheep house and becomes hot due to rising heat from the other decks of the sheep house and radiant heat from its metal roof. Rams experienced 7.35% mortality, and most of these mortalities (110/159) were due to heat stress. The rams were also housed on deck 11, the proportionally higher mortality in the rams relative to the lambs is likely due to their body weight to surface area ratio which reduces their ability to dissipate heat and lower body temperature.

9.2.4 Salmonellosis treatment trial

The cumulative mortality observed in each group following the initiation of treatment is presented in figure 9.8. Mortality was highest on the day following initiation of treatment and was not different between groups (7 mortalities per pen).

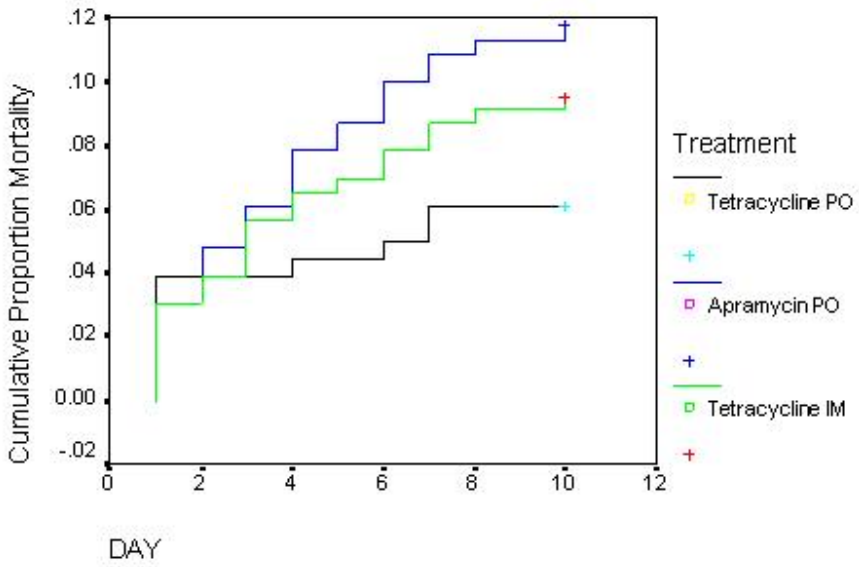


Figure 9-8 Cumulative mortality by day during antibiotic treatment trial for each of the three treatments

There was no statistical difference observed between the groups in regards to the proportion of sheep that died or in regard to the time to death. There was however a trend for the oral tetracycline to reduce mortality. An improvement in the appetite and demeanour of this group was also evident. The lack of statistically significant differences between groups in part reflects the limited sample size. With 150 animals per group a 10% difference in outcome is required to be statistically significant.

Previous investigations LIVE 112 (2002) concluded that antimicrobial agents should not be used therapeutically in live export sheep. These recommendations were based on anecdotal reports regarding lack of effectiveness and concerns regarding the potential for antimicrobial treatment to adversely affect gastrointestinal flora and favour antimicrobial resistant salmonella. Interestingly antimicrobial resistance was found to be very uncommon in the salmonella isolates recovered from sheep that died on ship. Antimicrobial use also has implications regarding drug residues in food producing animals.

The results of this limited trial suggest further shipboard investigation of therapeutic options is warranted for lines with clinical salmonellosis and high mortality. It would be worth setting up a protocol outlining the criteria for initiating pen based treatment and the specifics of the treatment to be administered. Unfortunately in this instance the dose of apramycin evaluated was the prophylactic dose and not the therapeutic dose. If such a trial is conducted it would be worth monitoring the antimicrobial resistance of bacterial isolates to facilitate interpretation of the results.

9.3 Summary of findings

Enteritis and inanition continue to contribute significantly to mortality in sheep exported live by sea, accounting for over 76% of diagnosed mortality

In earlier studies (Richards, Norris et al. 1989) inanition alone was shown to cause 43.4% of deaths and enteritis was shown to cause 20.2% of mortality. In this study, enteritis was found to be the most common cause of mortality (34.4%), followed by inanition (23.9%) and enteritis/inanition (18.2%).

Heat stress deaths were largely confined to a small number of voyages

Heat stress was recorded as the cause of death for 9.5% of mortalities diagnosed however; heat stress deaths were largely confined to a small number of voyages. Heat stress was not observed on 11 of the 19 voyages studied and accounted for less than 5% of total mortality on 6 voyages. Heat stress events tend to occur during the height of the Middle Eastern summer (July-August). The highest risk periods for heat stress mortality are times of high temperature and humidity which can occur at the equator, in the Straits of Hormuz and in port in Muscat (Oman).

Marked heat stress events were noted during voyages 8 and 17 (both July voyages) with heat stress responsible for 10.3 % and 17.1% of total voyage mortality. Voyage 17 was loaded from the east and the west and was accompanied by a project member. The heat stress event was recorded on day 21 as the ship entered the Persian Gulf (the Straits of Hormuz). For voyage 8 (loaded from the west), heat stress was recorded on day 13, most likely while the ship was entering the Gulf.

Pneumonia occurs sporadically and was recorded as the cause of mortality in 7.9% of diagnosed mortalities. Pneumonia mortalities tend to occur toward the end of the voyage and are typically bacterial in appearance. Bacterial pneumonia in sheep is caused by *Mannhiemia haemolytica*, a bacterium that is found in the upper respiratory tract of normal healthy sheep. Viral respiratory infection or stress lowers the animal's natural resistance to infection and allows disease to occur (Belknap 2002).

Other causes of mortality included miscellaneous diseases of sheep such as cancer, intestinal catastrophes (torsion or impaction of the gut), liver and kidney disease and a variety of systemic infections. These conditions account for almost 3.9% of mortality and are more often than not pre-existing conditions unrelated to the live export trade.

Trauma, urinary and clostridial disease together account for less than 2.5% of diagnosed mortality. Trauma deaths are more common during and immediately after loading and discharge. Urinary mortalities are similar to those occurring during assembly. Clostridial diseases are sporadic, fatal bacterial infections including enterotoxaemia and black leg.

Enteritis is the dominant cause of mortality early in the voyage with inanition and pneumonia contributing more significantly to mortality towards the end of the voyage

Cause of mortality varies with time but high mortality early in the voyage is generally maintained throughout the voyage

10 Development of an information management system

10.1 Introduction

Traceability of stock is required to assess consignment performance and for ongoing problem solving to facilitate continuing improvement in the industry. When LIVE.123 was initiated we needed to develop a data recording and management system to actively and passively collect consignment data at the assembly depots and on ship to investigate the performance of sheep consignments.

The development of this system involved a steep learning process regarding stock management in the industry, the availability of data and the logistics associated with data collection, recording and reporting. Visits to the assembly depots in Portland and Adelaide revealed that similar information was available at the different assembly depots. Two of the depots utilised Microsoft Excel© spreadsheets and the other two hand written dockets. When sheep are received at the assembly depot information regarding trucks and consignments were written in one or more locations depending on the assembly depot. Duplication in data entry was observed to occur between the sheep buyers, exporter and assembly depot. Effectively each step of the process collected similar information and recorded it into independent information management systems that ranged from docket books to excel spreadsheets. Data entry errors occur every time data is recorded. The potential for errors increases as the number of data entry points increases. No consistent format of data entry was employed within and across assembly depots which makes traceability more difficult. For example a vendor may be entered as Smith JB, Joe Smith, Outback Pty Ltd, etc. The PIC number provides for a unique traceable number, however the PIC's are long combinations of letters and numerals and transcription errors are common. There are also dealers who buy smaller consignments of sheep from multiple vendors resulting in lines of sheep with six or more PIC tags.

To promote the potential for ongoing passive data collection we embarked on the development of a modular information management system within which each module can function independently or as part of an integrated package. The assembly depot module was the first module developed to facilitate collection of accurate consignment data. During the first voyage the ship module was developed to record mortality records on ship. Refinements to both modules were made throughout the project according to feedback from industry. When the project was expanded to include consignments out of West Australia we had the opportunity to observe a vertically integrated system which provided useful insight as to the bigger picture of data requirements for sheep and business management.

Frustration with changing AQIS reporting requirements were evident hence we were careful to avoid developing a system that required duplication of efforts. The objective was to develop a system that would make the job easier, minimise data entry errors, incorporate error checking, and deliver reports that would meet AQIS requirements. To fully achieve this it became evident that the system needed to fulfil the sheep management and business needs of each component of the industry. The process needed to start with the exporter providing instructions to buyers and continue through to the ship. The sheep receival data also needs to link to the financial aspects of the business to enable the buyers to pay producers for the number of healthy sheep received. To this end we developed office, buyers, ship, and administration modules.

The implementation of this system differs across states and assembly depots with site specific adjustments tailored to assembly depot layouts and needs. There are three assembly depots currently using the assembly depot module and a fourth has modified their spreadsheet to provide

data in a compatible data format. In the eastern states the buyer data is inputted into Excel© by exporters and imported by the assembly depots.

The live sheep export business effectively provides sheep of different weights, sex, breed, and class according to the requirements of customers. The international importer provides specifications to the exporter who relays these specifications to their buyers along with the instructions required to meet AQIS and export requirements.

10.2 Administration module

The administration module is designed to meet the needs of the exporter in regards to providing buyers with access to the system, outlining buying instructions, monitoring the buying process, and managing all data files. The menu options in this module are presented in figure 10.1.

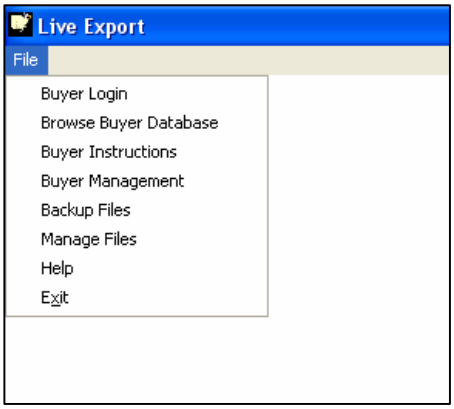


Figure 10-1 Administration module menu options

The exporter relays buying instructions to the buyers using the buyer instruction selection. This information is linked to the buyer module to give the buyers a running tally of the number of sheep purchased verses number required. The information entered in the buyers' instructions and the interface used to enter this data is presented in figure 10.2.

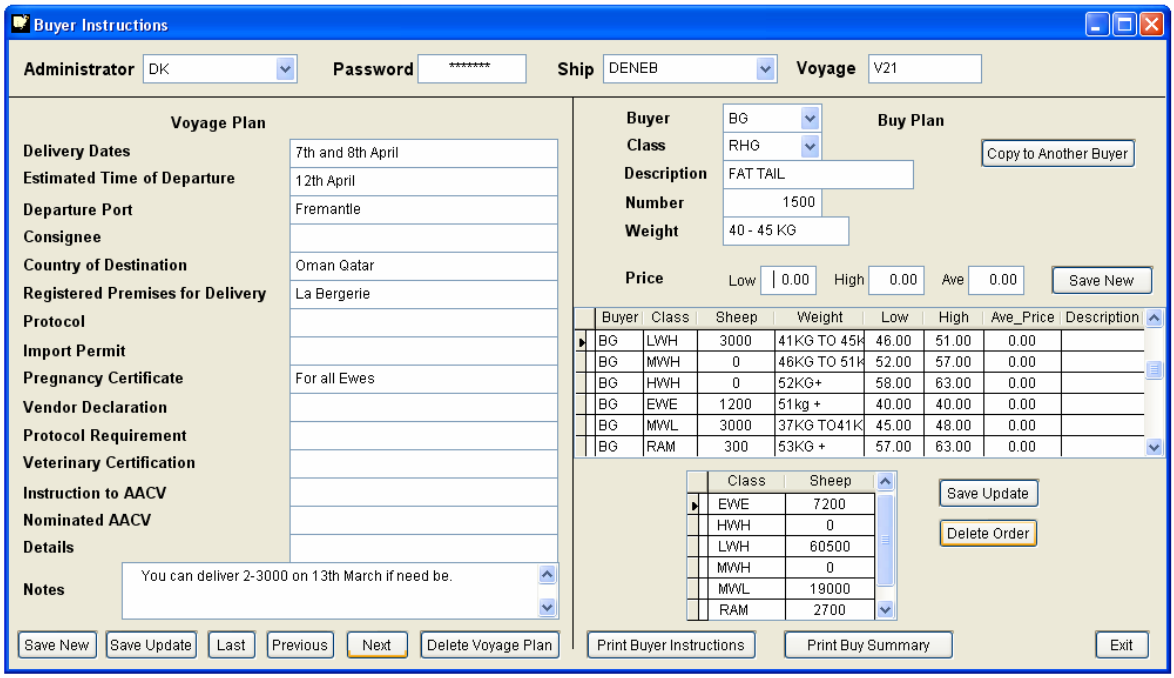


Figure 10-2 Administration module; interface for entering Buyers instructions

Buyers access the system remotely entering the details of the sheep they have purchased. The exporter can monitor the progress of all buyers at any time by logging onto the system and browsing the buyer database. This provides running totals for each class of sheep for the whole shipment and for each buyer (Figure 10.3).

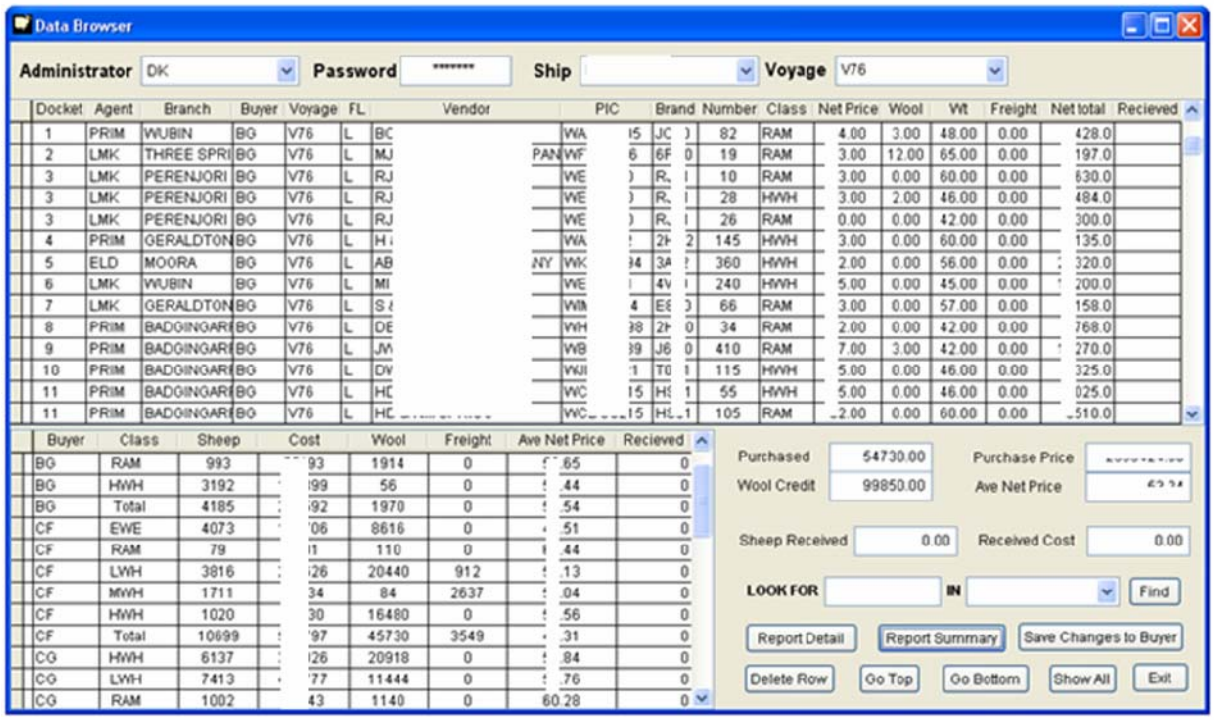


Figure 10-3 Administration module; buyer database browser showing running totals

10.3 Buyers module

The first point of contact between the producer and industry is the sheep buyer. Once the buyers have received their buying instructions they utilise their contacts to source sheep to meet the desired specifications. When the buyer purchases sheep they enter the vendor and consignment details into the system remotely. The information entered is accessible to the exporter and assembly depot. Previously, assembly depots entered the same data into their own systems with the associated transcription errors. With the integrated system the pressure is taken off the assembly depot weigh bridge as much of the required information has already been entered by the buyers and is directly accessible to the weigh bridge in the assembly depot. The assembly depot receival office subsequently plays an important part in error checking verifying consignment details including vendor, PIC, ear tags, sheep class and weight. Buyers enter data remotely via the internet; the data entry interface is presented in figure 10.4.

A feature of the system in West Australia is that it utilises data in the NLIS database. This is important for consistency of data entry and greatly facilitates data analysis by reducing the time required to match consignment and mortality records. To minimise data entry errors the buyer enters the vendors PIC to retrieve the vendor details and brand. As the PIC is entered matching PICS appear in a drop down list from which they can select the correct vendor. The advantage of this system is that it reduces the amount of typing required and associated typographical errors and

secondly it ensures that the data is recorded utilising a predetermined format that is consistent across buyers and shipments, facilitating traceability.

In the eastern States the buyers are not utilising the remote system. To avoid duplication of data entry, excel spreadsheets containing vendor details and consignment data are imported by the operators of the assembly depot.

10.4 Assembly depot module

A number of functions are carried out at the assembly depots; these can be broadly divided into receival, feeding, and loadout. Receival and loadout are busy times in the assembly depot with 20 – 100 thousand sheep entering or leaving the feedlot within a 24 to 48 hour period. Maintaining sheep throughput is important from a logistical perspective and for the health and well being of the sheep. There are two opportunities to collect and verify consignment details in the assembly depot, at the weigh bridge and at the unloading dock. Following the unloading of sheep the truck driver presents the National Vendor Declaration, buyer docket and the assembly depot unloading docket to the operator of the weigh bridge. The weigh bridge operator records the weight of the truck, checks the documentation, enters the number of sheep received and prints a receipt. Data accuracy is enhanced when the driver presents all of the appropriate documentation and when stockmen at the unloading dock check the identity of sheep and verify the ear tags present on the sheep match the NVD (the sheep identification recorded on the NVD is sometimes incomplete and match the identification present on the sheep). The buyer database is accessible to the assembly depot so they can select the vendor from the list rather than having to re-enter data. The PIC (West Australia) or Buyer Docket Number (Victoria and South Australia) are utilised as relational data fields. The data entry interface is presented in Figure 10.5.

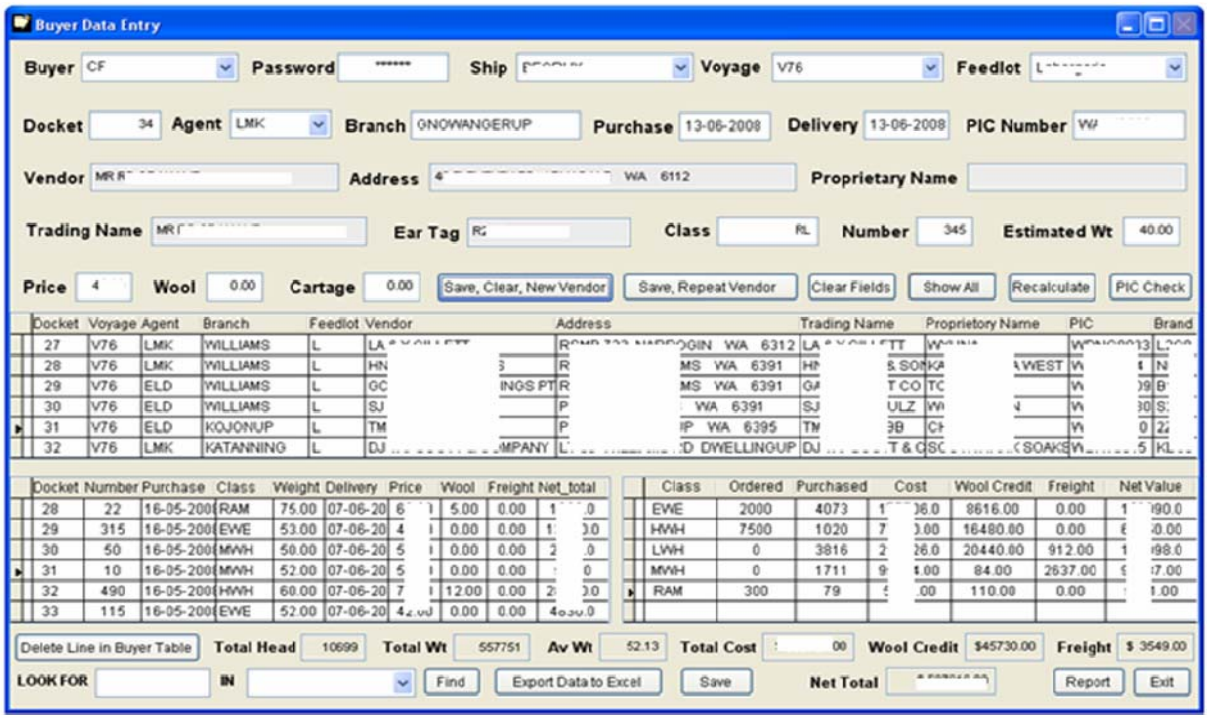


Figure 10-4 Buyer module; data entry interface

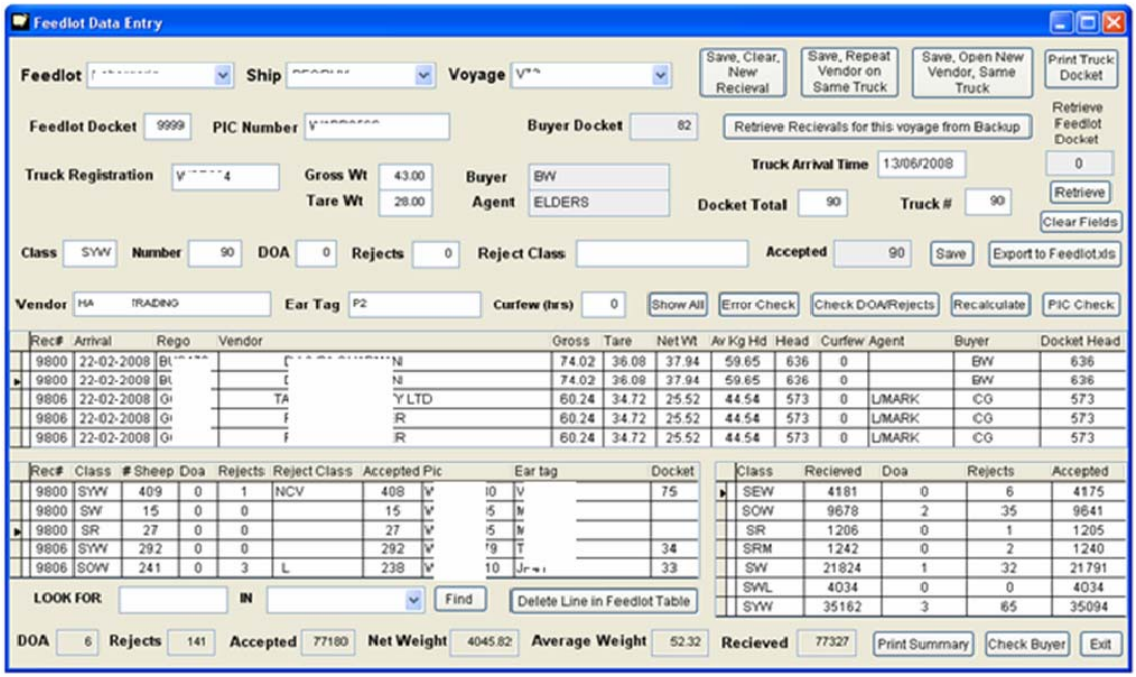


Figure 10-5 Assembly depot module; receival data entry interface

Running totals of the number of sheep received in total and by class are maintained. The operator can also search the buyer database and cross check for outstanding receivals and discrepancies. The search function is useful for retrieving vendor details and NVD's for AQIS ear tag checks.

As sheep are drafted into lines they are transferred into sheds or paddocks. From a problem solving perspective it is desirable to record paddock usage and paddock outcomes to determine if there are patterns of disease morbidity and mortality. AQIS also conduct an assembly depot inspection during the assembly period. To facilitate the tracking of sheep through facilities and to provide stocking records to meet AQIS requirements a paddock stocking option is included in the feedlot module for the Victorian and South Australian Depots. This module is under development for the West Australian depot. An example of the data entry form used to record the stocking information is provided in figure 10.6.

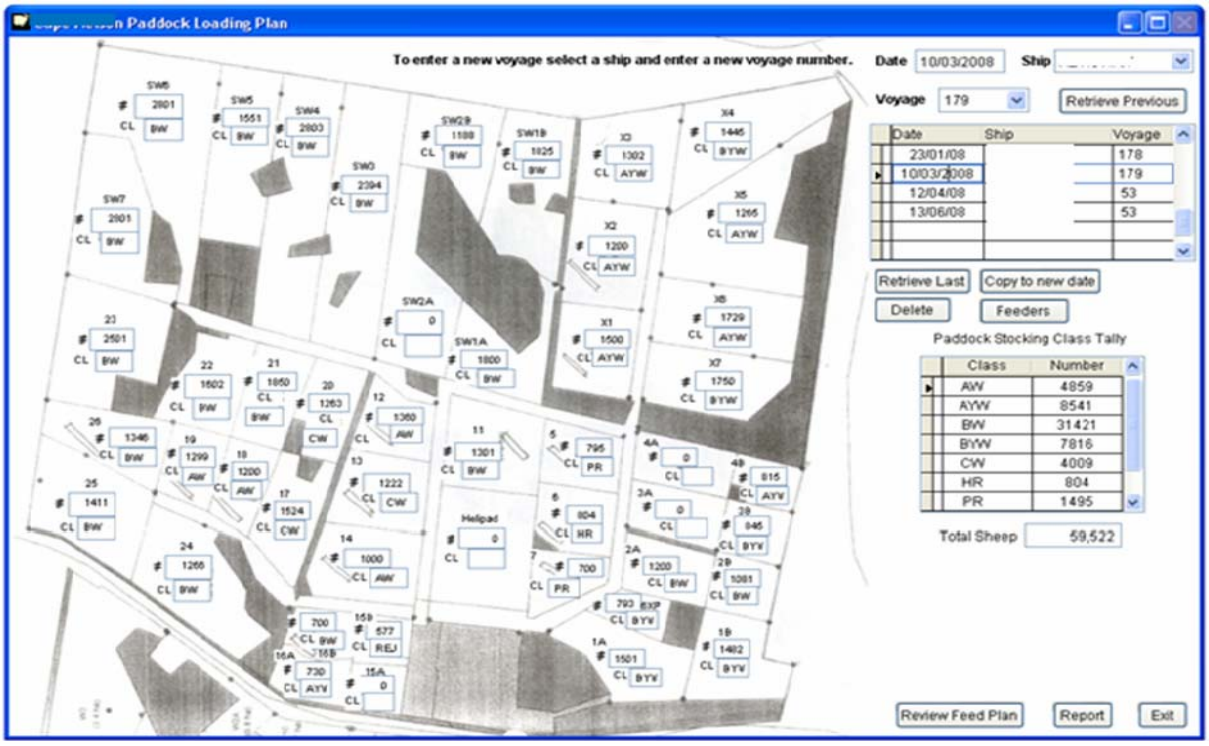


Figure 10-6 Assembly depot module; paddock stocking interface

The paddock stocking plan reports provide details regarding the capacity of each paddock, number and class of sheep, and any changes in stocking during the assembly period. The stocking plan is also linked to the feeding program with the number of sheep in each paddock utilised to calculate the daily feeding requirements according to the proposed feed plan which is outlined at the onset of the assembly period. The feed plan dictates the number of kilograms of pellets and hay to be fed per head per day for the duration of the assembly. The data input form for the feeding schedule is presented in figure 10.7.

Mortality records are recorded during the assembly period. A data recording form and database has been created to facilitate the recording of assembly mortality and to link mortality records with consignment and paddock databases. The data entry form is presented in figure 10.8.

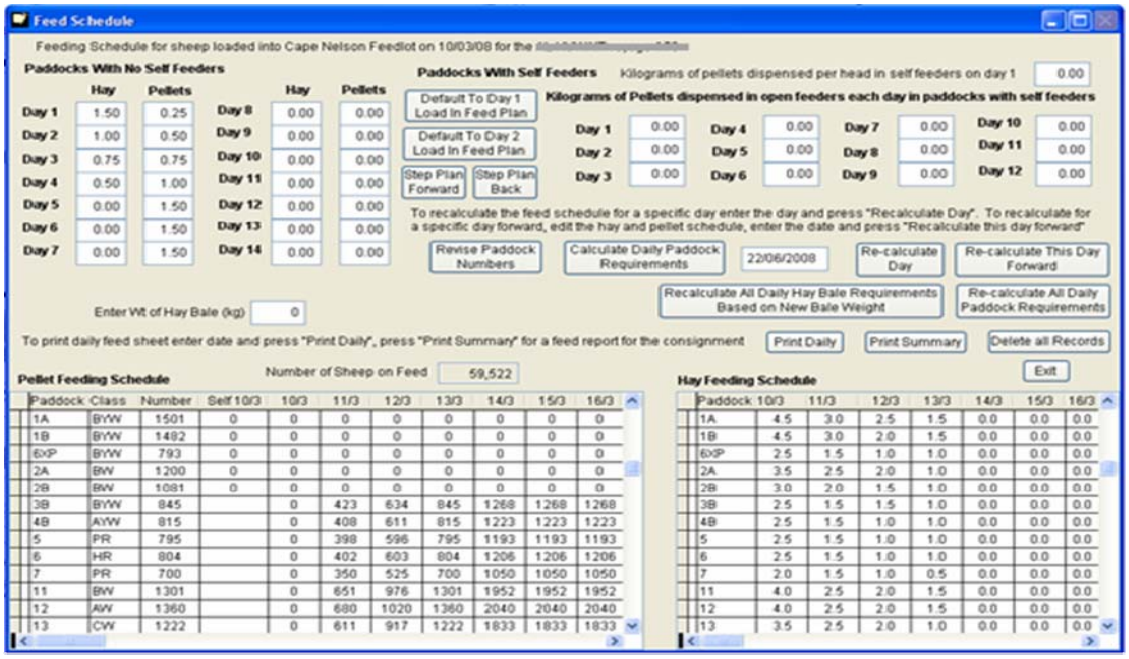


Figure 10-7 Assembly depot module; feed plan

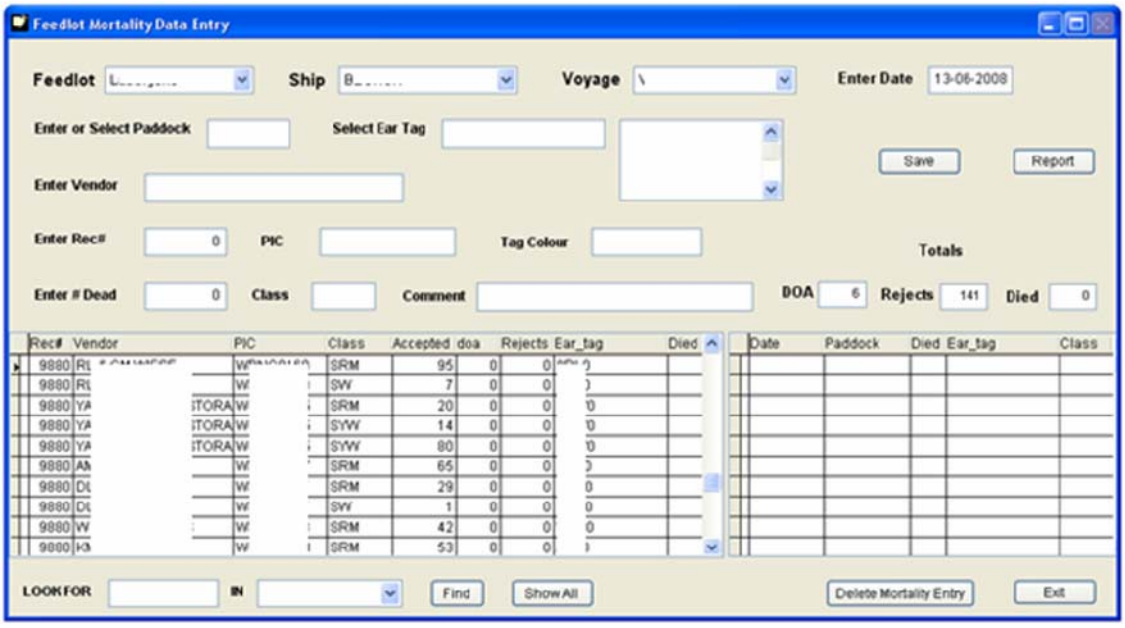


Figure 10-8 Assembly depot module; mortality recording

At the end of the assembly period sheep are loaded onto trucks to be transported to the wharf. The loadout count and weights are utilised to correctly load the sheep according to the load plan report generated by the Heat Stress Risk Assessment Model (HotStuff). Sheep weight and number are important variables in the Hot Stuff model so it is important to collect accurate weights as sheep leave the assembly yards. The data input form for this portion of the assembly module is presented in figure 10.9. This module also compares the wharf counts to the assembly counts to provide a measure of the discrepancy between the two.

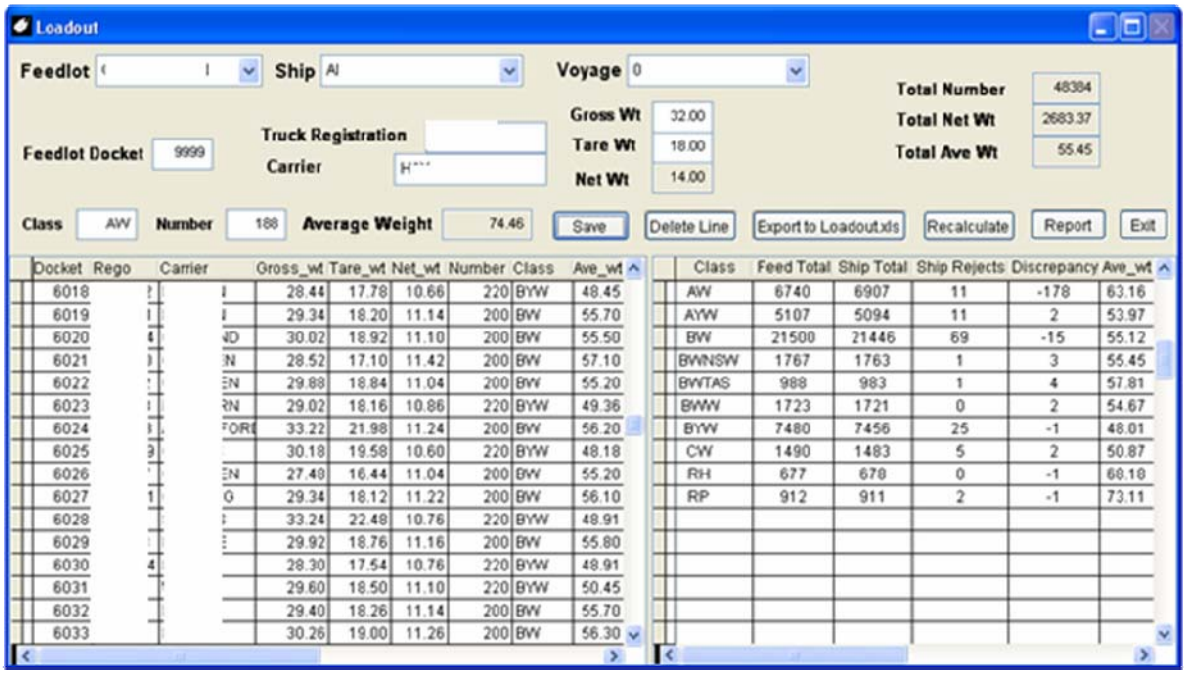


Figure 10-9 Assembly depot module; loadout data entry interface

10.5 Ship module

To facilitate the collection of mortality data on ship a standalone module was developed to record daily mortalities. It is not uncommon to find sheep with more than one ear tag, having a list of vendor names and ear tags is helpful for matching dead sheep to a vendor. For example when a sheep has more than one tag you record the details of the tag that matches the consignment database. Sometimes sheep are reported to have one tag but within the line there is a mix of tags. If more than one animal dies it is sometimes possible to identify these lines as sheep in the same pen may have a common tag which is different to the recorded tag. The assembly module has an export option for transferring sheep identity and line numbers to the ship and the ship module has an import option for receiving assembly data.

During the voyage veterinarians may conduct post mortem investigations. During the accompanied voyages we conducted several hundred post mortems. To document gross pathological changes we collected digital images of post mortems conducted at the assembly depots and on ship. For a large library of images it is useful to have a method of image retrieval. To this end the daily mortality

recording form has an option for linking images to a case for future retrieval. The data recording form utilised on ship is presented in figure 10.10.

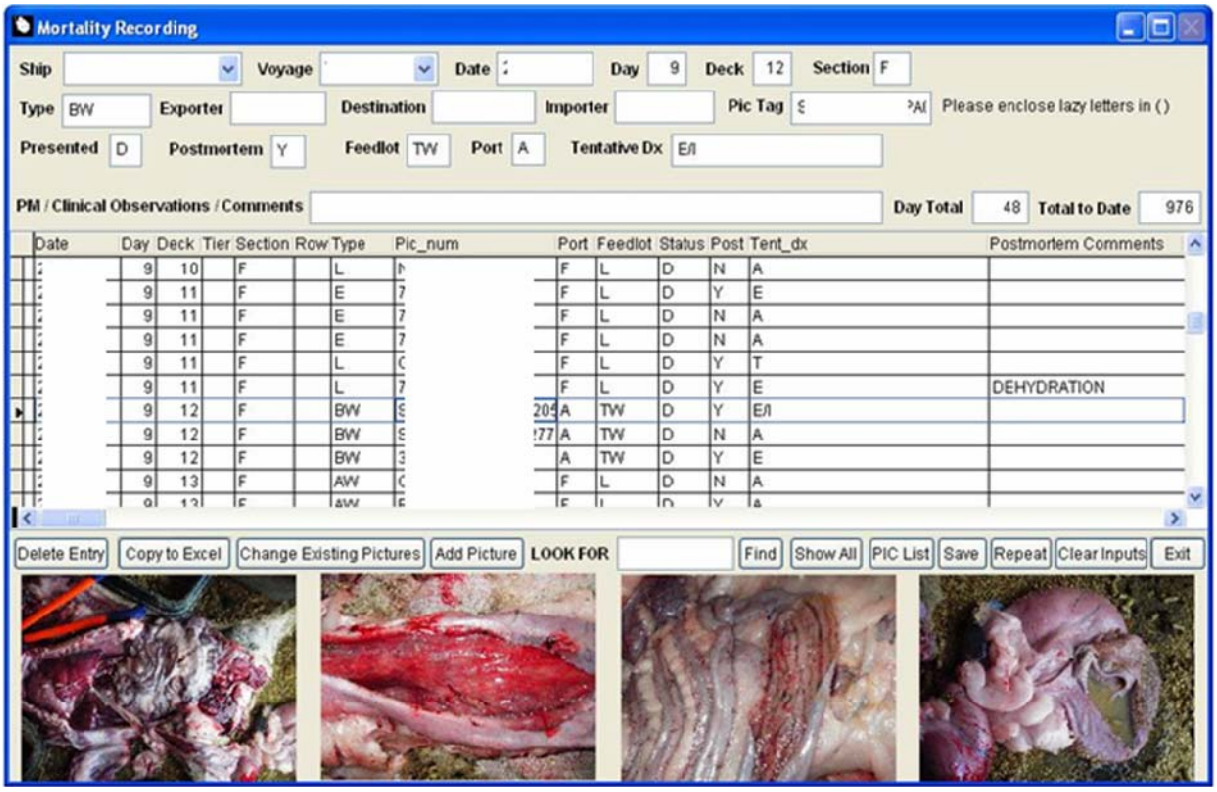


Figure 10-10 Ship module; data entry interface

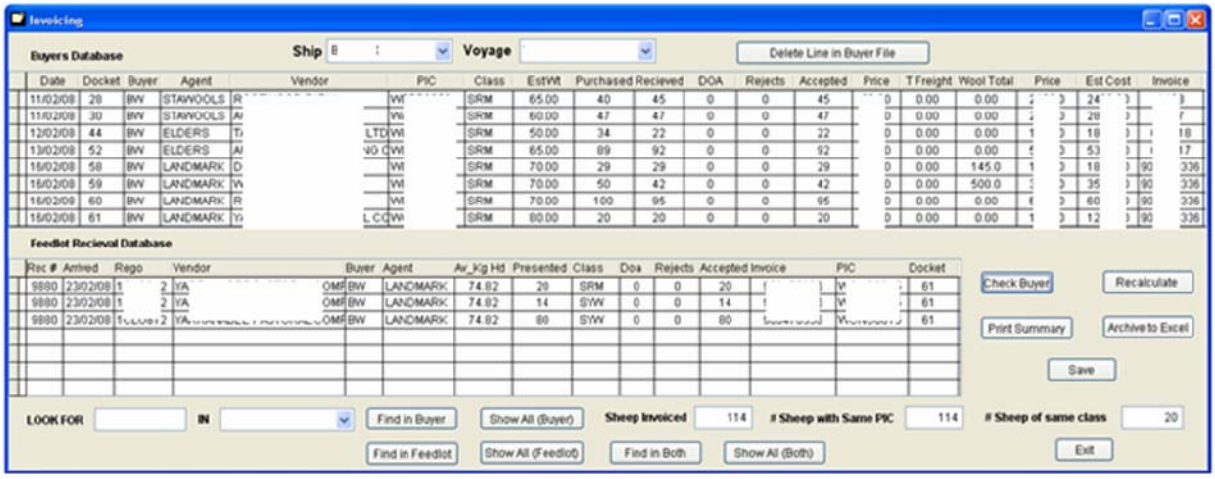
Reports can be generated to tabulate mortality by day, deck and class for the whole shipment or for sheep loaded from specific ports.

The ship module also includes options for recording details of the load plan, ship loading, and unloading. The reporting options are designed to simplify the generation of the veterinarians end of voyage reports. Adoption of the ship module was variable. Two of the regular veterinarians installed the program on their laptop computers. Other “fill in” shipboard veterinarians generally utilised data recording log books that we supplied

10.6 Office module

An information management system that does not incorporate financial aspects of the business is unlikely to be adopted by industry because it will require duplicate data entry for business management. An underlying objective in developing this system was to promote industry adoption by making a system that promotes good management and good business. To this end an office module was developed to simplify the process of correlating invoices with sheep receivals prior to payment. The office module also produces a number of reports that can be used to review sheep costs and buyer performance. An example of the invoicing interface is presented in the figure 10.11.

Figure 10-11 Office Module; invoicing interface



11 Success in achieving objectives

Objective 1: *Determine the rate, causes and predisposing factors of mortality for live export sheep and lambs at the different stages of the live export supply chain (to the port of discharge).*

Objective 2: *Determine the relative mortality risk for sheep and lambs by region and time of year, including whether or not pastoral sheep and lambs are more at risk for mortalities than sheep and lambs from non-pastoral areas.*

Objectives 1 and 2 were achieved successfully and conclusions are presented in Section 13. Receival and assembly period mortality was recorded and analysed for a total of 35 shipments. Voyage mortality was recorded and analysed for 27 shipments. Post-mortem examinations were carried out during 3 assembly periods and 5 voyages.

Objective 3: *Determine the relationships between inappetence and salmonellosis incidence at the pre-export assembly depot and on ship as related to on-farm, transport, seasonal and regional factors.*

Objective 3 was partially achieved. Energy balance and salmonella studies were carried out during the assembly periods. Metabolic indicators of negative energy balance were used as a proxy for detecting groups of sheep that were not consuming sufficient feed to meet their nutritional needs. The relationships are outlined in detail in section 7 and conclusions are presented in Section 13. Mixing of sheep at receival means that it is not possible to investigate property of origin, transport or sheep factors that may be contributing to inappetence and salmonellosis in the assembly depot. For this reason objective 3 was only partially achieved. While it is possible to compare the voyage outcome for sheep assembled at different assembly depots with the current management systems and systems of sheep identification it is not possible to evaluate the performance of sheep assembled in different paddocks or sheds within an assembly depot as the identity of sheep in each location is unknown.

Objective 4: *Formulate additional strategies for producers and exporters that can be applied prior to arrival at the pre-export assembly depot to minimise the level of inappetence, salmonellosis and other conditions that affect the health of live export sheep and lambs delivered to Portland and Adelaide, as well as sheep exported from Perth in split shipments.*

Objective 4 was not achieved. Higher mortality lines were most notable in the latter half of 2007. A large proportion of the mortality was attributable to a small proportion of vendors. In this regard it was notable that sheep specifically sourced from Broken Hill and sheep originating from Queensland were at increased risk of mortality. In regards to finding answers for producers from Broken Hill further research is required. Given the fact that pastoral sheep sourced from South Australia did not have an increased risk of mortality it appears that the mortality risk observed for NSW pastoral sheep relates more specifically to a regional phenomena rather than the fact that they are pastoral sheep per se. Project team members travelled with consignments of these sheep and investigated high mortality consignments. In each case the consignments experienced outbreaks of salmonellosis.

It was notable that pastoral sheep sourced from NSW had an increased risk of mortality whereas pastoral sheep sourced from South Australia did not. This suggests the mortality risk is related to a regional phenomena rather than pastoral status per se. In regards to finding answers for producers from the western division of NSW further research is required. Project team members travelled with

consignments of these sheep and investigated high mortality consignments. In each case the consignments experienced outbreaks of salmonellosis. Interestingly these sheep often presented with full rumens particularly at the start of the outbreaks suggesting that inappetence was not necessarily the predisposing factor. It is also notable that in the Human population of Broken Hill lead toxicosis is recognized as a human health problem that is associated with the soil type and mines (Lyle et al. 2006). Chronic exposure to lead is known to be immunosuppressive (Fernandez-Cabezudo et al. 2003). It would be interesting to investigate the heavy metal status of sheep from the properties that experienced high mortality to determine if a similar problem is present in the sheep population of this region.

Objective 5: *Develop a working prototype computerised information management system to record health and mortality data on sheep during all stages of live export and formulate a detailed project proposal for development of a final working version of this system.*

Objective 5 was achieved successfully. The computerised information management system is in operation and has been used successfully by members of the research team, assembly depot operators and on-ship veterinarians. At the completion of this project the program was in use at two of the eastern assembly depots and one assembly depot in West Australia. The development of this system has been an evolving process, initially identifying needs and subsequently refining and developing components to improve capacity and functionality. People within industry who are comfortable with computers have welcomed the concept. A lot of the information collected is proprietary so the system has been developed for exporters to maintain within their own business. The uniformity of the data structure makes it simple to collate data for analysis. As assembly depot managers, buyers, exporters, and veterinarians utilise the system they appreciate the potential for automation of data collation and reporting. If the industry at large wishes to adopt an information management system it is anticipated that this process is likely to be ongoing requiring the need for ongoing support.

12 Impact on meat and livestock industry

The conclusions and recommendations of the LIVE.123 project are aimed at improving sheep health and welfare through reducing the occurrence of the most common disease processes (salmonellosis and inanition) and ultimately reducing mortality.

12.1 Impact now

Implementation of the recommendations is likely to reduce mortality in the live sheep trade for sheep exported from the eastern states.

Improving the health and welfare of the live export sheep has many potential benefits for industry. The direct benefit of improved health and welfare is increased profitability for direct stakeholders associated with a reduction in sheep losses. In addition to lowering mortality, reduction in the occurrence of disease (morbidity) would increase the proportion of sheep delivered in optimum health, continuing Australia's reputation for supplying excellent quality livestock. Improved health and welfare and a reduction in mortality over any time frame is essential to improving public perception of the trade and ensuring sustainability.

12.2 Impact in five years time

The most profound benefit to industry is ensuring the long term sustainability of the live sheep trade. In 2005, sheep exported live by sea represent 12% of all sheep turned off farms in Australia and it is estimated that if the trade were closed gross value of production in the sheep and lamb sector would be $220 million less per year (Hassall 2006).

Pursuit of research and development recommendations has the potential to achieve more substantial improvements in health and welfare and greater reductions in mortality. Over the next five years, maintaining this commitment will definitively demonstrate the livestock export industry's ongoing commitment to improving animal health and welfare.

Published mortality rates are frequently used to criticise the industry. As described above, 76% of mortality is related to salmonellosis and/or inanition and the overall voyage mortality rate was 0.88%.

Based on these numbers, controlling salmonellosis and inanition could reduce overall mortality to as little as 0.2%, even if half of the mortalities associated with salmonellosis and inanition were prevented mortality would drop to 0.5%. Mortality of 0.5% or less would approximate natural mortality rate for all farmed sheep in Australia (3-5% per annum, or 0.25-0.41% per month). Ongoing improvement is essential for the sustainability of the trade.

13 Conclusions and recommendations

13.1 Mortality rates and causes

13.1.1 Receival

Deaths on receival were almost exclusively attributed to trauma and included limb fractures and smothering. The overall rate of rejection was 47.9 per 10,000 sheep and the overall rate of receival mortality was 6.5 per 10,000 sheep. Compared to young wethers, the relative risk of mortality during road transportation was 1.84 times higher for old wethers, 2.14 times higher for rams and 1.98 times higher for sheep from the pastoral zone. Distance travelled to the assembly depot also had a significant effect on risk of mortality. Compared to sheep in category 1 (travelling less then 200km), the relative risk of mortality was twice as high (2.07 times) for sheep in category 4 (500-799km) and more than three times higher (3.38) for sheep in category 5 (>800km). There was also an association between death during transport and transport injury (journey rejection). The relative risk of mortality, in receivals with journey associated rejection was twice as high (1.99 times) as that in receivals without journey associated rejection. Increasing body weight was significantly associated with mortality, for each 1kg increase in body weight, the risk of mortality increases by 1%. Unexpectedly, curfew time was not found to significantly impact mortality during road transportation.

A large volume of road transport data was collected during this study. This data could have application in providing background performance data as industry reviews road transport guidelines. An important variable that should be considered if livestock are to be unloaded and rested during long haul transport is the implications of this practice on mortality (loading and unloading carry injury risk) and on the risk of promoting salmonellosis. Rest yards could become a nidus for salmonella exposure and lead to an increase in post transport morbidity and mortality.

13.1.2 Assembly period

Assembly period mortality was found to be low and sporadic in the absence of a salmonellosis outbreak. One salmonellosis outbreak was studied in assembly depot 6 with a mortality rate of 0.55% (55 deaths per 10,000 sheep). The overall mortality rate for the eastern assembly depots was 0.05% (5 deaths per 10,000 sheep). Causes of assembly depot mortality after receivals included salmonellosis, urinary tract disease and miscellaneous diseases.

Recommendation 1. Intervention to reduce assembly depot mortality is not indicated in the absence of a salmonellosis outbreak.

During this study period salmonellosis was reported more frequently in West Australian assembly depots than eastern Australian and was observed to occur more frequently between August and October. Studies of sheep assembled in West Australia were initiated during 2007 so these comments are based on shipboard observations during 2005 and 2006 and from assembly depot observations and post mortems in 2007. In 2001 and 2002 the eastern states assembly depots were reported to have more problems with salmonellosis than the west and similar carry over mortality was reported on ship. The assembly depot sampling conducted in this study illustrated that salmonella contamination of the assembly depot yards is common and the in-vitro studies illustrate that it is likely that the challenge dose will fluctuate in this environment. Currently the assembly depots have no means of assessing salmonella challenge risk or proven method of managing this risk should it be identified.

Recommendation 2. Develop tools to rapidly measure salmonella contamination in the environment.

It would be useful to develop a methodology to rapidly and simply quantify the number of salmonella present in the yards. Such a methodology would enable industry to evaluate risk and evaluate methods of pathogen reduction. These cyclic problems are likely to continue unless risk assessment and risk management interventions are developed.

Although outbreaks of salmonellosis in assembly depots are infrequent, salmonellosis is the most common disease problem during the assembly period and when outbreaks occur the consequences can be dramatic. There are currently no clear science based recommendations describing the most appropriate way to mitigate the health and welfare consequences of a salmonella outbreak. From the perspective of sheep welfare the industry is leaving itself open to criticism.

Recommendation 3. Review the literature regarding approaches to manage salmonellosis in sheep and develop science based best practice guidelines for each segment of the live sheep trade.

13.1.3 Voyage

Salmonellosis and inanition continue to be the predominant causes of mortality with enteritis the leading cause (34.4%), followed by inanition (23.9%) and enteritis/inanition (18.2%). In many of the mortalities diagnosed as enteritis/inanition, there was evidence of adaptation to the export ration so enteritis is hypothesised to be the primary disease process. The relative proportion of mortality attributed to enteritis and inanition in this study is very similar to previous studies. In this study cause of mortality was primarily attributed to enteritis if a sheep was found to have reduced rumen fill (pellets) accompanied by signs of acute inflammation of the gastrointestinal tract. Discussions with Dr Tony Higgs (Department of Agriculture and Food, WA) suggest that in previous studies a similar sheep would have been classified as a primary case of inanition. The relative difference in the proportion of mortality attributed to salmonellosis and enteritis may in part reflect a difference in case definition.

Sheep entering assembly depots have been held off feed for variable periods of time and have variably compromised immunity. Salmonella contamination of the yards provides a challenge with variable numbers of salmonella. There are many variables that are likely to impact the immunity of sheep and the number of salmonella in the environment. In the univariate analysis long curfews (> 48 hours) were associated with an increased risk of voyage mortality. Curfew times were not available for all lines and this variable ultimately dropped out of the multivariate analysis. In discussions with people within the export industry we also observed different opinions regarding the appropriate curfew time for sheep prior to road transport. There is a difference in opinion between exporters from eastern and West Australia which may in part reflect the consequences of differences in truck design. East coast buyers recommend longer curfew times than buyers in West Australia. Sheep trucks in West Australia provide for better drainage of faeces and urine than trucks in the eastern states.

Recommendation 4. Conduct research to provide robust guidelines regarding curfew times that account for differences in truck design.

13.1.4 Risk factors for mortality

There is an increase in mortality in Winter and Spring

Results of previous studies have led to the development of the hypothesis that time of year has a significant effect on sheep appetite drive and metabolism and subsequently performance and mortality on ship (Higgs, Norris et al. 1991). In addition to this, Kelly (1996) observed increased mortality during periods of high temperature and humidity, as are encountered during the Middle Eastern summer from July to September. This increase in mortality was not due to heat stress deaths but to an increase in other causes of mortality, primarily inanition (Kelly 1996).

62.7% of lines had no mortality
74% of mortality was traced to 18 % of lines

This is in agreement with previous studies that found mortality rates in sheep exported live by sea vary widely between farm groups, with certain lines of sheep suffering significantly higher mortality than others. Norris et al. (1989) found that 54% of all deaths during export occurred in only 25% of lines. In addition to this, Higgs et al. (1999) found that mortality rates in different farm groups ranged from 0 to 28.2%, with half of all sheep that died being traced to 14.2% of consignments.

Old (full mouth) wethers have a higher mortality risk than lambs

This is also in agreement with previous studies that found different classes of sheep vary in their ability to adapt to sea transport. Higgs et al. (1991) analysed 50 shipments and found lower mortality rates in wether hoggets (0.52%) than adult wethers (1.63%).

The data available indicate sheep sourced from the pastoral zone in NSW and Queensland sheep carry an increase risk of mortality. Pastoral sheep sourced from South Australia did not have an increased risk of mortality.

Recommendation 5. The guidelines in the standards regarding pastoral sheep should be adjusted to exclude NSW pastoral sheep from the trade between May to October and to allow South Australian pastoral sheep during the same period.
Recommendation 6. The disparity in mortality risk observed between NSW and South Australian pastoral sheep should be further investigated as it provides an opportunity to elucidate causes of high mortality risk. Such an investigation may also provide insight as to possible approaches to reducing mortality risk.

NSW pastoral sheep have a higher risk of mortality than South Australian pastoral sheep and sheep from non-pastoral areas

Sheep from Queensland have a higher risk of mortality than sheep from other states

Queensland sheep represent sheep purchased from Queensland by a southern vendor and later sold to live export. Higher mortality lines were most notable in the latter half of 2007. A large proportion of the mortality was attributable to a small proportion of vendors.

The mortality risk observed in Queensland sheep and sheep from pastoral zones in NSW could not be attributed to distance travelled to reach the assembly depot as this variable was not found to have a marked effect on the risk of mortality

13.2 Persistent inappetence, salmonellosis and inanition

13.2.1 Relationships

Sheep are exposed to pathogenic salmonella organisms in the assembly depot, with the yards an important site for exposure. Environmental factors are important to salmonella exposure with warm wet conditions allowing salmonella to proliferate in the assembly depot, increasing the risk of salmonellosis. While inappetence can lead to salmonellosis, there are other factors including level of immunity and magnitude of salmonella challenge that influence whether or not clinical salmonellosis will occur.

The investigation into the relationship between inappetence and salmonellosis identified factors which can be manipulated to minimise salmonellosis and potentially reduce mortality. Salmonella infection can be minimised either by decreasing salmonella challenge, increasing resistance to infection or both.

13.2.2 Inappetence and the export ration

The results of postmortem examinations suggest that the current export pellet ration is of good quality. Despite evidence of negative energy balance occurring in the assembly depot, the overall occurrence of inanition was found to be lower than reported in previous studies (accounting for 23.1% of mortalities rather than the 43.4% reported by (Richards, Norris et al. 1989)) indicating that fewer sheep are failing to adapt to the ration. In addition to this, there was no evidence of gastrointestinal disease related to the ration, more than 5,600 post-mortem examinations were performed during investigation, and no cases of ruminal acidosis were recorded.

Pellet dust was observed to accumulate in feed troughs to a greater or lesser extent during different voyages. The policy of the ships chief officer appeared to be a driving factor in this area. Ruminants generally do not like dusty feed and intakes will be favoured by avoiding the build up of pellet dust.

Recommendation 7. Research methods to assess pellet integrity and develop guidelines for feed assessment and presentation.

In regard to inappetence on the ship it was noted that all of the feeders are positioned such that the sheep have to put their head through to the outside of the pen. In cattle feedlots the concept of shy feeders is recognised and facilities often include stanchions to cater for shy feeders by providing less intimidating access to feed.

Recommendation 8. That research be undertaken to determine if provision of access to feed on the inside of the pen would reduce the incidence of shy feeders.

Dry matter intake is influenced by access to water. If water is limiting livestock tend to eat less. Cleaning of water and feed troughs is part of the daily routine on ships. From a labour perspective cleaning troughs is relatively labour intensive. Crew often switch off the waters during the evening to facilitate cleaning in the morning.

Recommendation 9. That research be undertaken into stock water delivery systems to reduce leaks (leaking troughs are switched off) and facilitate the ease of cleaning (i.e. simple for the crew to drain troughs without wetting the pens).

13.2.3 Prevention of salmonellosis

13.2.3.1 Decrease exposure to salmonella

The assembly depot sampling conducted in this study illustrated that salmonella contamination of the assembly depot yards is common and the in-vitro studies illustrate that it is likely that the challenge dose will fluctuate in this environment. Currently the assembly depots have no means of assessing salmonella challenge risk or proven method of managing this risk should it be identified. These cyclic problems are likely to continue unless risk assessment and risk management interventions are developed (See Recommendation 2.)

Scraping assembly depot yards to remove the build up of faecal pack should help to reduce the potential for proliferation of salmonella in these facilities. According to the results of in-vitro experiments the risk is likely to be greater during the winter and spring when there is a high throughput of sheep, fresh pack, and moist conditions.

Recommendation 10. Applied research in assembly depots is required to provide more robust recommendations as to the best protocols to employ to manage salmonella contamination. The product of recommendation 2 would facilitate this process.

During the assembly period “problem” mobs are brought into the yards to be drafted. Common “problems” in the assembly depot include pink eye, salmonellosis and failure to eat. Stockmen were observed to be astute in their judgement of identifying “sick” sheep and conversely selecting healthy sheep during an outbreak of salmonellosis in an assembly depot. The only problem with this strategy related to the use of common yards for sorting sick sheep and receiving new sheep. Sick sheep amplify salmonella contamination, shedding 10^9 salmonella per gram of faeces. Where infrastructure changes are planned or feasible, building a separate set of yards for receival, drafting “problem mobs”, and load out would promote a unidirectional flow of sheep and help to reduce carryover of virulent salmonella serotypes to subsequent assembly periods.

Recommendation 11. Develop biosecurity guidelines for assembly depots that include discussion of facility design and disease prevention strategies.

13.2.3.2 Increase immunity to infection

Resistance to salmonella infection is a function of innate and acquired immunity. Innate immunity is natural immunity to infection and is determined by the genetic make-up of the animal and normal organ function. Innate immune mechanisms can be compromised by many things including altered rumen function, dehydration, stress, and other diseases. Acquired immunity develops following exposure to a pathogen with the animal producing an immune response specifically directed against the pathogen. This process stimulates immunological memory and the capacity for a rapid response to subsequent pathogen exposure.
During the course of this study a number of examples of differences in host immunity were observed. As previously described 18% of lines accounted for 74% of mortality. Specific examples of differences in immunity to salmonella were observed during the assembly period and on ship. During the intensive assembly period sampling conducted in 2005, “Young Pastoral Wethers” and “Non Pastoral Young Wethers” were mixed in the same paddock. Salmonella faecal shedding was

significantly greater in the “Non Pastoral Young Wethers” than the “Young Pastoral Wethers” despite the sheep occupying the same paddock. Similarly on another shipment a group of distance sheep were mixed with non-distance sheep. During this voyage the sheep travelled well until day 8, at which time an outbreak of salmonellosis was observed in the distance sheep. During the outbreak in these pens disease was limited to distance sheep. A potential precipitator for the outbreak was wetting of the faecal pack from hosing the open deck above the sheep house. Regardless of the precipitating factor the difference in outcome for the different lines of sheep was striking, during this and a subsequent outbreak in pastoral sheep on another voyage the affected sheep presented with acute inflammation of the small and large intestines and rumens full of pellets. The degree of rumen fill in these high mortality events (13 – 22.6% mortality) suggested that inanition was not the main predisposing factor. The cause of the differential host immunity was not readily apparent.

Vaccines are utilised to stimulate these acquired immune mechanisms. This type of immunity takes weeks to develop limiting practical application in the live sheep trade. The salmonella vaccine currently on the market in Australia for cattle also has a limited spectrum of activity so it will not be effective against the mix of salmonella serotypes that sheep may be exposed to in the trade. It has been shown that the DNA Adenine Methylase attenuated salmonella vaccines can stimulate innate immune function and can provide protection against salmonella infection within 24 hours of administration. This type of vaccine can be administered in drinking water and could potentially be a practical, low cost and effective way to minimise salmonella infection and reduce mortality in the live sheep trade.

Prevention of salmonellosis would reduce mortality in the live sheep trade. Traditional salmonella vaccines are unlikely to be effective in providing protective immunity to sheep. To be effective in this industry a vaccine needs to be simple to administer to avoid induction of additional stress and increased yarding, efficacious across a range of serovars, and cost effective. DNA adenine methylase live attenuated vaccines may be able to meet these criteria in sheep. Experiments in mice, poultry, and calves have demonstrated cross protective immunity against a diversity of salmonella serotypes using a single vaccinal strain, induction of enhanced immunity within 24 hours of administration, and ability to induce effective immunity following oral delivery. Recently we have evaluated oral delivery of this vaccine in adult sheep and have found it to be an effective way of delivering the vaccine. It is envisaged that it may be possible to incorporate the vaccine into drinking water during the first 24 hours of the assembly period. Further work would be required prior to application in the industry.

Recommendation 12. Investigate the potential application of salmonella vaccines in the live sheep trade.

13.2.4 Management of acute salmonellosis

Only one outbreak of salmonellosis was observed during an assembly period between October 2005 and December 2007. During this assembly period 55,000 sheep were in the depot and only 35,000 were required for the voyage. Careful drafting, segregation and selection effectively stocked the ship with healthy sheep that did not experience high voyage mortality. During different voyages we observed different pens of sheep experience high morbidity and mortality salmonellosis events. Different veterinarians implemented different management strategies for sick sheep ranging from no treatment, changes in diet, individual animal medication and medication of pens via drinking water (Recommendation 3).

13.2.5 Information management

During the course of this project several assembly depots, and exporters have utilised the information management system developed for this project. While the adoption of this system is not uniform across industry the participation by a number of the key stakeholders does provide an opportunity for problem solving. While this system is functional it is not refined and industry stakeholders are continuing to explore the potential for enhancement and integration of flock and business management. Broad application of such a system across the industry places the industry in a strong position to address industry wide and voyage specific questions

During the course of this project we consulted with exporters, assembly depot operators, sheep buyers, veterinarians, stockman, and ship's crew to develop a prototype information management system.

During the project we accompanied two voyages that had exporter consignments that exceeded 2% mortality requiring a report to AQIS. In both cases we were able to provide the exporter with a report providing the details regarding which sheep died and where they had come from. This information was helpful to clarify what had happened and what contributed to mortality during these shipments.

When problems occur there is a great demand for information but the industry will only reliably have this information if data collection is routine. In a similar fashion if you want to know what the causes of mortality are in consignments that experience high mortality the ship board veterinarian needs to know the number of sheep in each consignment, their identity, have a pre-defined trigger to prompt investigation and have resources available to support the investigation. We utilised digital cameras to collect images of every post mortem conducted in the assembly depot and on ship.

Recommendation 13. Develop mortality investigation guidelines for stockman and ship board veterinarians

Recommendation 14. Develop an industry wide information management system to facilitate problem solving.

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15 Appendices

15.1 Mortality classification

15.1.1 Enteritis

Enteritis in sheep can be caused by a number of infectious agents. The most common cause in lot-fed sheep as well as the live export trade is salmonellosis. Enteritis in adult sheep may also be caused by clostridial disease, Yersiniosis, Johne's disease, campylobacter and parasitic infections.

Acute enteritis is 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. It is not uncommon to also see signs of septicaemia including marked congestion of the mesenteric vessels. In more chronic enteritis, lymph nodes are still large but the intestinal lesions are less severe, including typical 'tiger stripe' lesions in the caecum.

The majority of enteritis diagnoses are associated with salmonella infection. Infrequently, clostridial enteritis is also seen. Clostridial enteritis has similar gross changes to salmonella enteritis. Changes in clostridial enteritis are more haemorrhagic with little enlargement of mesenteric lymph nodes and rapid decomposition of tissues (Navarre and Pugh 2002).

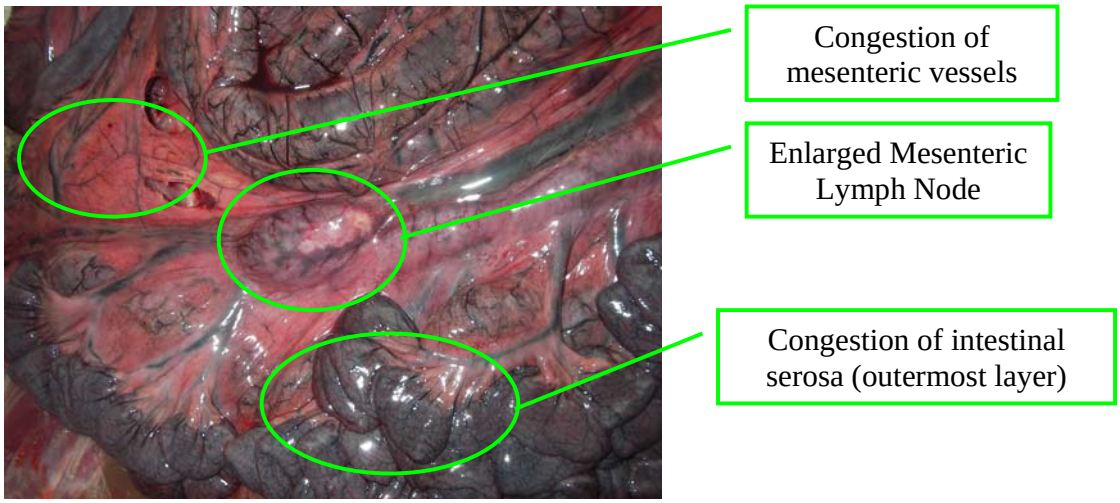

Figure 15-1 Acute enteritis

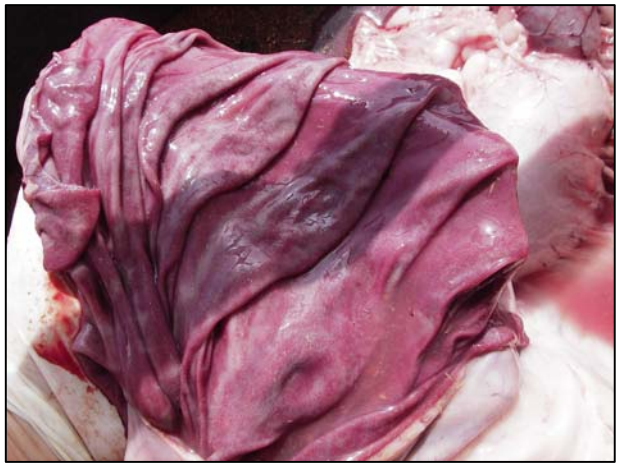

Figure 15-2 Acute inflammation of the abomasal mucosa

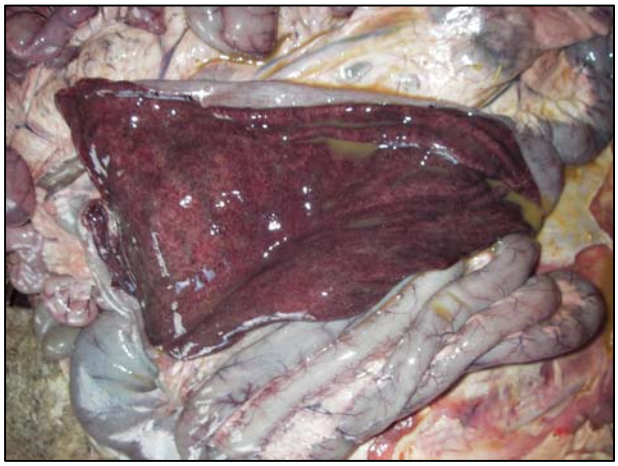

Figure 15-3 Acute inflammation of the intestinal mucosa

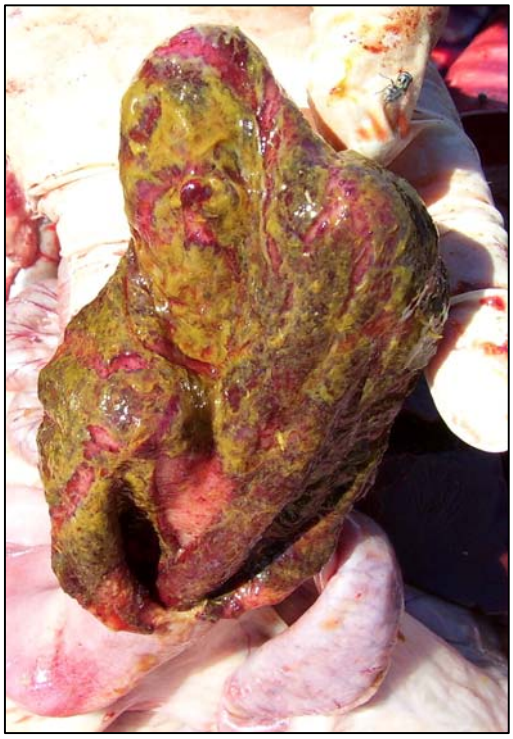

Figure 15-4 Severe acute inflammation of the caecum

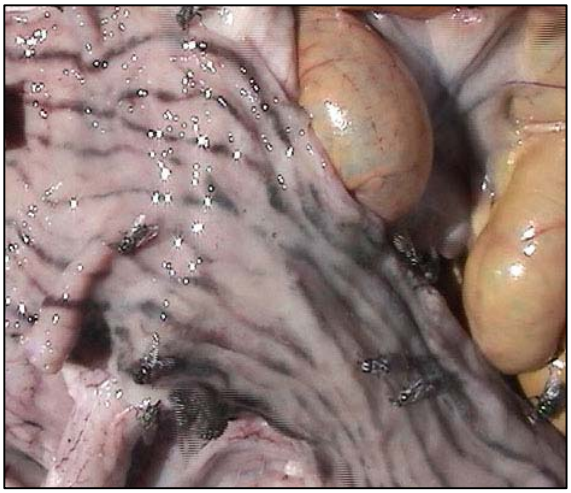

Figure 15-5 Chronic inflammation of the intestinal mucosa

Formalin fixed Intestinal segments were collected from a number of postmortem examinations for histopathology. Typical lesions of salmonella enteritis include loss of intestinal villi, infiltration of the lamina propria with inflammatory cells, microthrombi and crypt abscesses.

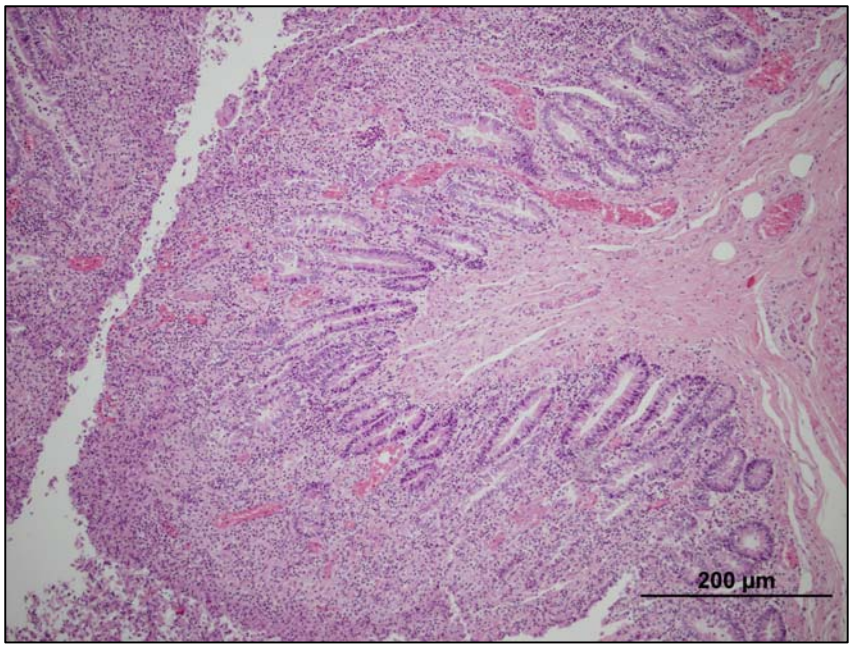

Figure 15-4 Low power view of intestinal segment showing loss of villi, microthrombi and infiltration of inflammatory cells

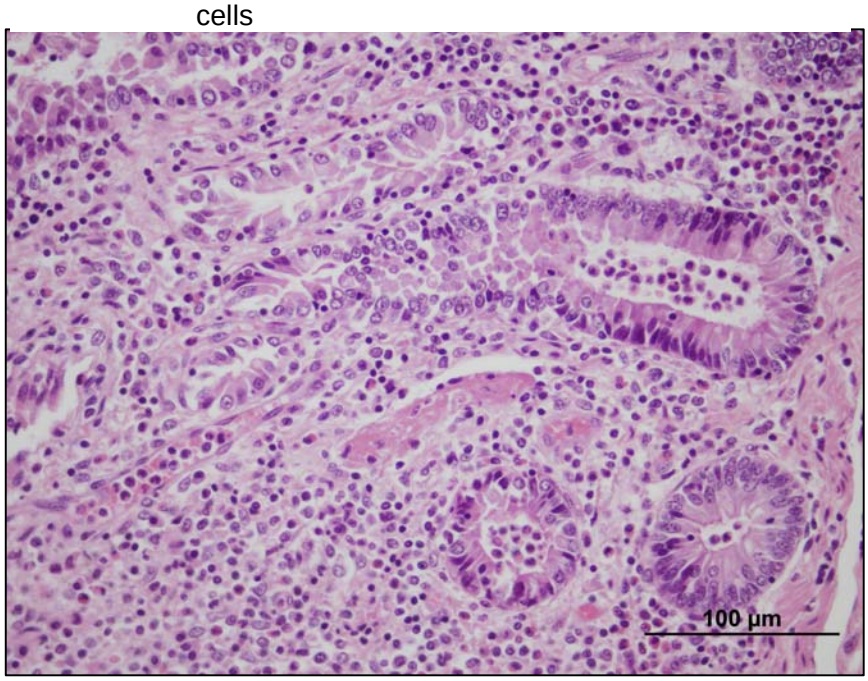

Figure 15-5 High power view of intestinal segment showing crypt abscess

15.1.2 Inanition

Inanition is characterised by very low or absent rumen contents, poor body condition and an absence of other significant pathology (i.e. gross enteritis or pneumonia). The condition is characterised by a reduction in rumen solids; often the rumen contents are predominantly liquid. Enlargement of the gall bladder is common and in fatter sheep it is not uncommon to see evidence of fat mobilisation and accumulation of fat in the liver.

Figure 15-8 Rumen with no solids

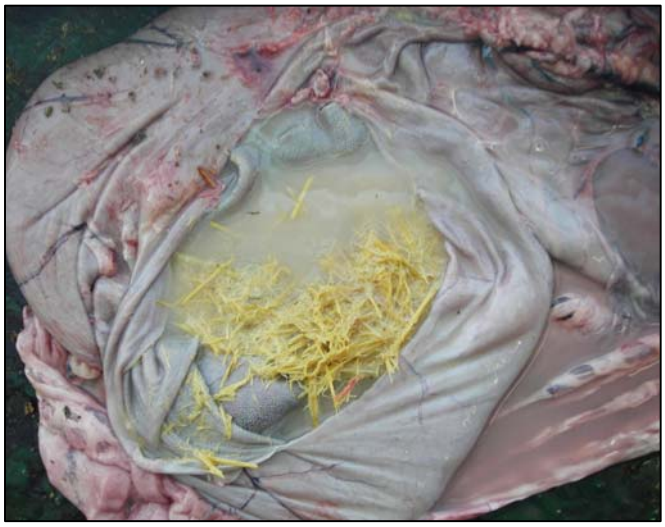

Figure 15-9 Rumen with low solids

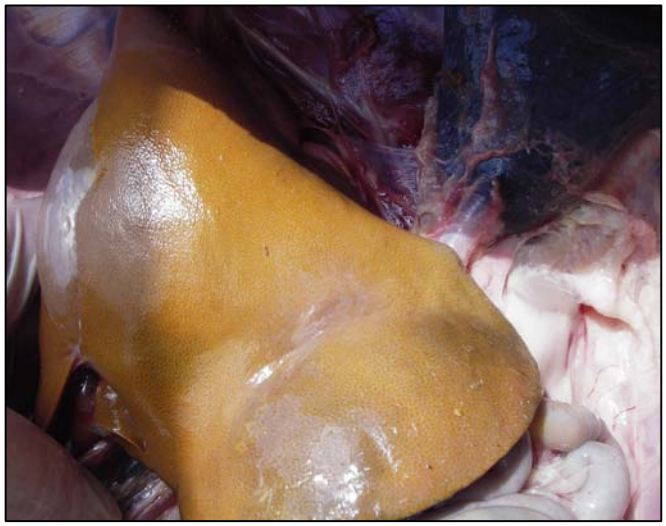

Figure 15-6 Fatty liver

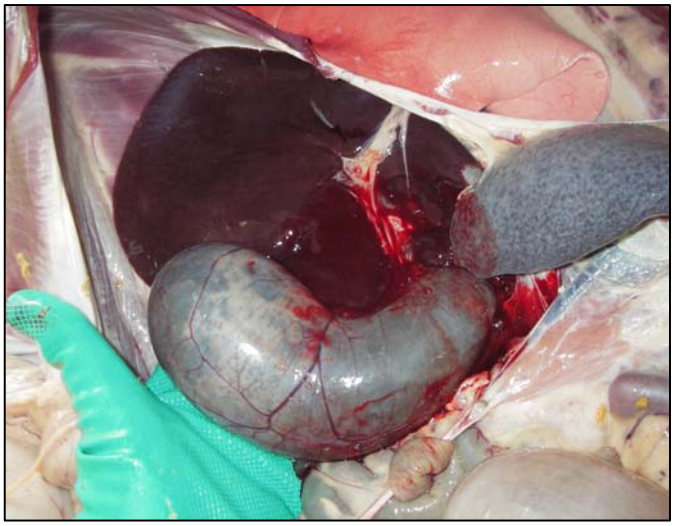

Figure 15-7 Enlarged gall bladder

15.1.3 Enteritis/inanition

In this category a combination of the gross changes described under the enteritis and inanition categories is seen. 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. It is hypothesised in these cases enteritis was the initiating disease which lead to inappetence and later inanition.

15.1.4 Pneumonia

Pneumonia is characterised by deposits of inflammatory clots in the chest cavity and consolidation (inflammation and hardening) and congestion of the lung tissue.

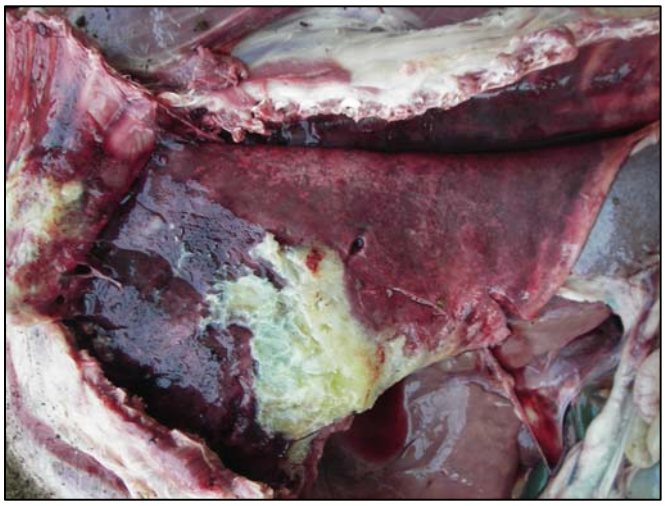

Figure 15-8 Pneumonia with fibrin clots attached to the pleural surface

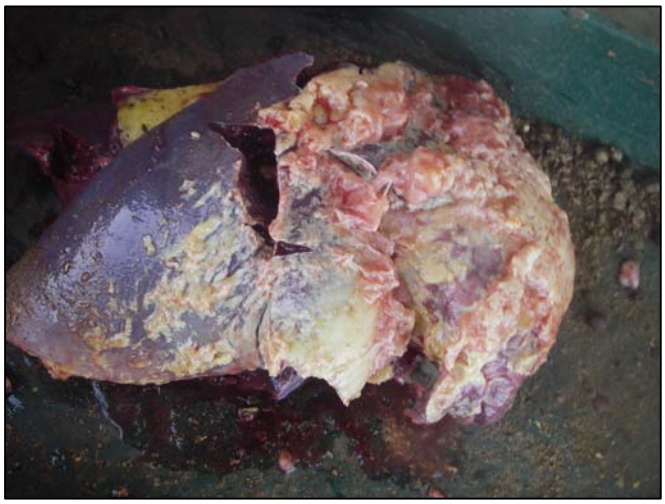

Figure 15-10 Pneumonia with consolidation of the lung and extensive fibrin clots

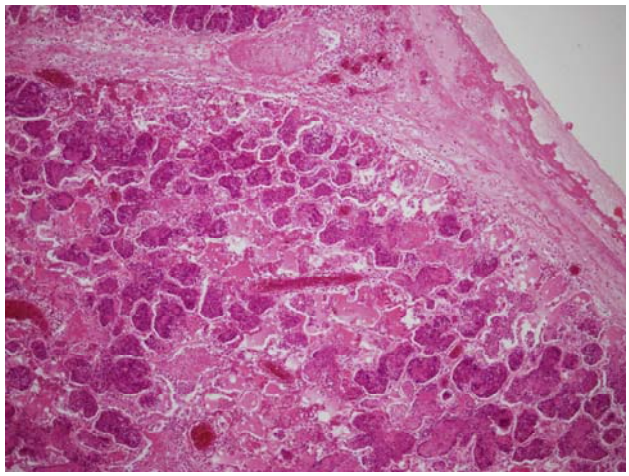

Figure 15-9 Fibrinosupprative pleuropneumonia

15.1.5 Trauma

Mortalities in the trauma category include sheep with fractures, spinal injuries and those that have gone down on truck and been trampled. Often these animals will be alive at the time of receival and are identified and euthanased by stockmen. Sheep with spinal injuries may show some or all of the following signs; partial or complete paralysis, knuckling of the lower limbs and abnormalities or in-coordination of gait. Sheep that have been trampled are invariably down, stained and show difficulty breathing. On post-mortem examination there may be bruising of the tissues below the skin, fractures of the ribs, rupture of internal organs (liver, spleen, gastrointestinal tract), congestion (blood accumulation) in the lungs and accumulation of froth and/or feed in the large airways. Feed in the large airways indicates that the animal regurgitated and then aspirated (breathed in) feed from the rumen.

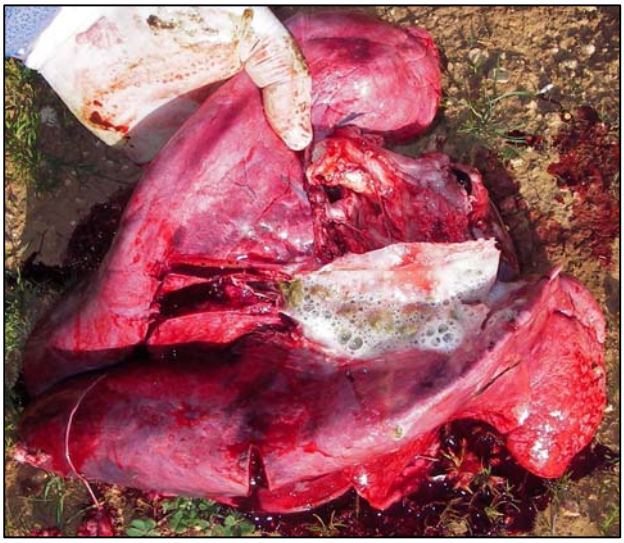

Figure 15-15 Froth and feed in the trachea

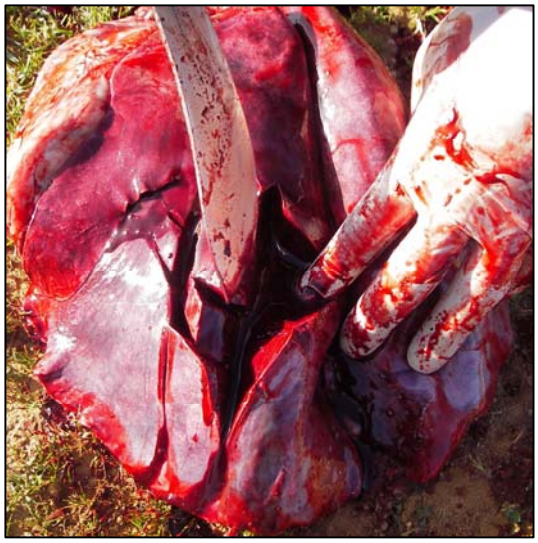

Figure 15-16 Congestion of the lung

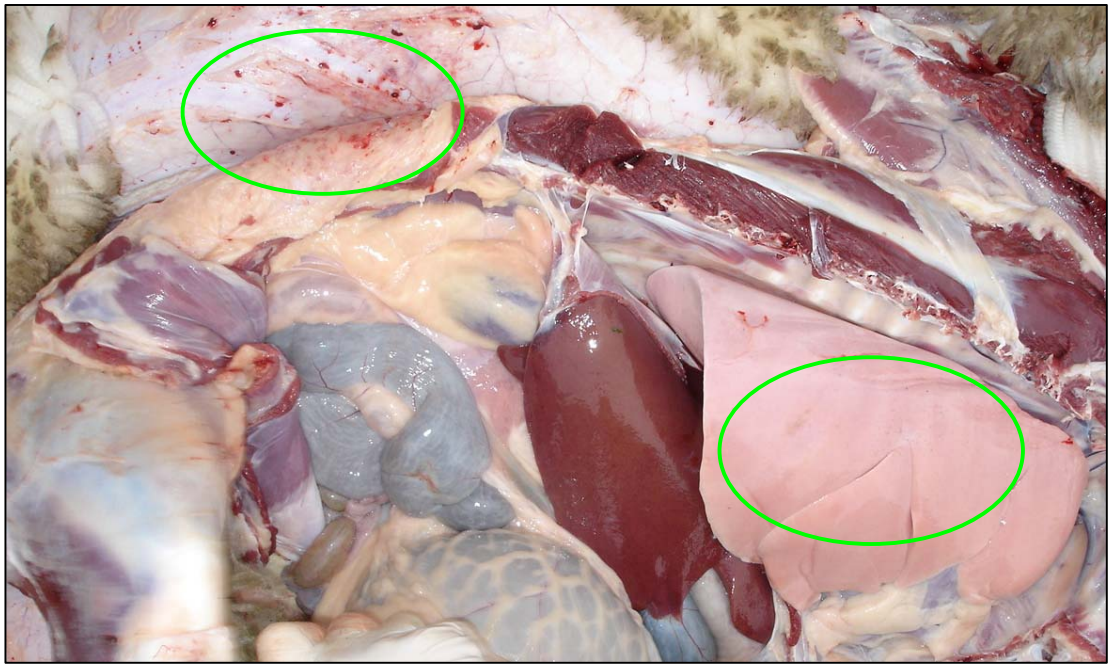

Figure 15-11 Carcase with bruising under the skin and normal lungs

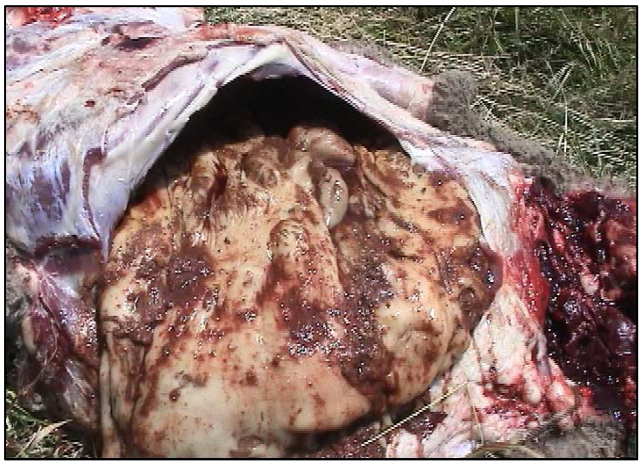

Figure 15-18 Rupture of the gastrointestinal tract with spillage of feed into the abdomen

Figure 15-19 Bruising under the skin

15.1.6 Urinary

Post-mortem examination changes seen with urinary obstruction and rupture include rupture of the urinary tract (usually at the bladder), inflammation (reddening) of the bladder, leakage of urine into the abdomen and stones in the urethra or kidney. Leakage of urine from the bladder leads to the accumulation of urine in the abdomen, blood vessels lining the peritoneum and the surface of abdominal organs may appear dark and engorged. The urinary tract may also rupture outside the abdomen. The urethra may rupture under the abdominal skin, when this occurs there will be accumulation of fluid under the skin of the abdomen and usually swelling of the pizzle. The accumulated fluid smells like urine.

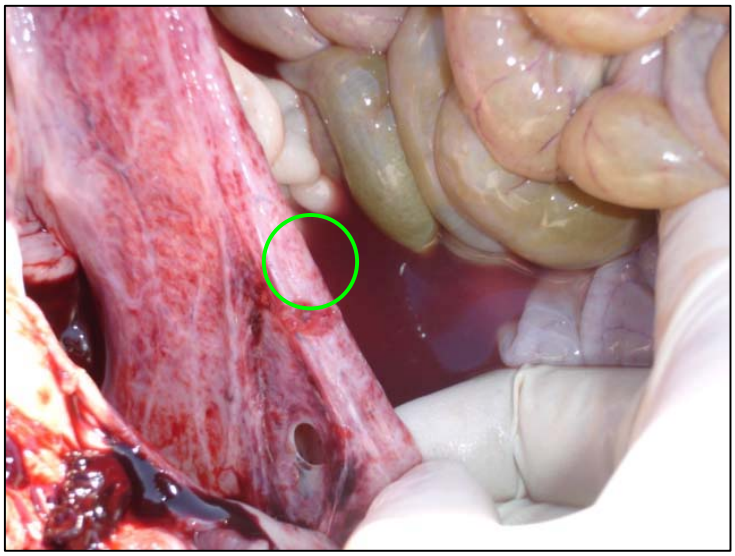

Figure 15-12 Urinary rupture, with inflammation of the bladder and blood and urine in the abdomen

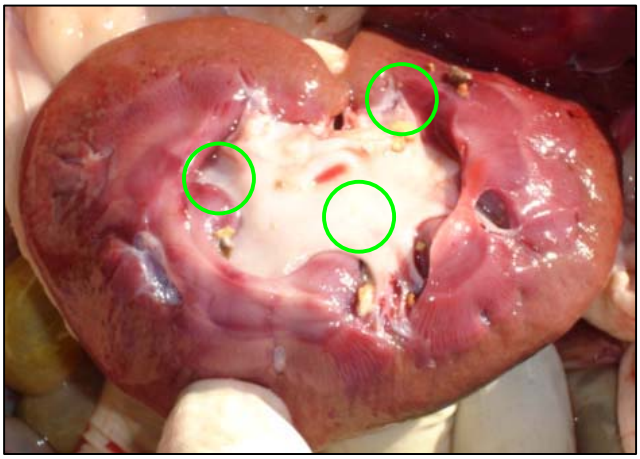

Figure 15-13 Calculi (stones) in the kidney

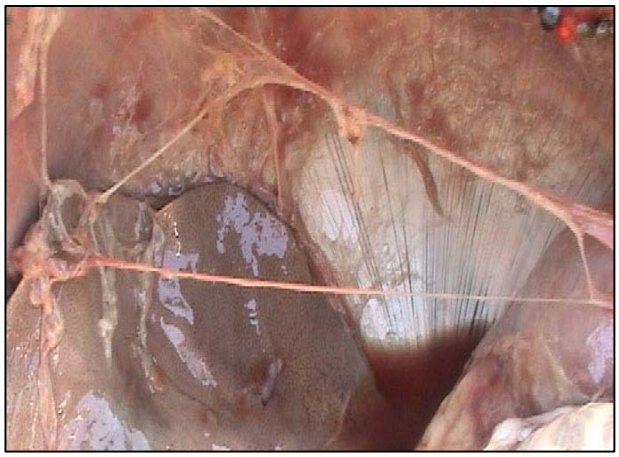

Figure 15-14 Peritonitis with fibrin in the abdomen

15.1.7 Other

Mortalities in the other category include several miscellaneous causes of mortality. These diseases were observed less frequently and included peritonitis, neoplasia, clostridial disease, polioencephalomalacia, and intestinal torsion. Many of the miscellaneous cases of mortality are likely to be related to pre-existing conditions and not the export process.