CHAPTER 6

EPIDEMIOLOGIC STUDIES INTO SALMONELLOSIS IN THE LIVE SHEEP TRADE

6.1 Introduction

Epidemic salmonellosis has long been associated with intensive sheep management systems, and had been described before in the live sheep trade (Chapter 1). The VOY1 to VOY3 studies (Chapter 4) showed that salmonellosis mortalities began early in the export process then declined during the voyage. The disease appeared to be exacerbated by low feed intake in the feedlot. It also occurred more frequently in groups of sheep with indications of stressful trucking en route to the feedlot, and in those which lacked prior experience with hay feeding. However, key questions remained about the nature and origin of the epidemics as they occurred in the live sheep trade.

Three separate studies were performed, all aimed at elucidating the epidemiology of salmonellosis in the trade. The first observational study described the patterns of faecal excretion of salmonellae in a cohort of sheep going through the export process. The second study investigated the patterns of salmonellosis mortalities in the feedlot phase using large scale, necropsy studies. The third study examined long term trends in the salmonella types present in the trade. It involved retrospective examination of seven large collections of salmonella isolates that had been obtained over five years during the research program.
6.2 Materials and Methods

6.2.1 A Description of the Patterns of Faecal Excretion of Salmonellae

A cohort of 600 adult wethers was monitored for faecal excretion of salmonellae throughout the export process on research voyage VOY4. This was the voyage, introduced in Chapter 5, from which the nine case studies of the inanition syndrome were drawn. It was the voyage which had an extended feedlot period due to a shipping accident. The cohort comprised 100 sheep from each of six farms which had committed to supply sheep to the VOY4 consignment. The farms were chosen by convenience based on their proximity to the Regional Veterinary Laboratory at Hamilton. On each farm, 100 sheep were selected for the cohort from the larger group which were being consigned. The large group was mixed in yards and 100 were simply drafted out. All selected sheep were identified with two ear tags.

The presence or absence of salmonellae in the faeces of each sheep was determined at twelve intervals throughout the export process. Blood samples were also collected at the same intervals, as described in Chapter 5. The sampling began one day prior to the sheep leaving their farm of origin, and continued intensively through the feedlot period (Days 4, 5, 6, 7, 9 and 12), and at sea (Days 17, 20, 24, 28 and 32). Shipboard samplings were staggered, because of space and labour limitations, with one third of the cohort being sampled each day in a 3 to 4 day rotation. Faeces were collected by hand from the rectum of each sheep, or if no faecal pellets were present, a rectal swab was taken. Specimens were handled and cultured, and salmonellae were identified, as described in Chapter 2. All isolates of *Salmonella Typhimurium* and *S. Bovis-morbificans* were phage typed.

The temporal patterns of excretion for each type of salmonella were established using incidence rates. The rates were calculated for each interval between consecutive
samplings by dividing the number of new cases of faecal excretion for a particular type of salmonella, by the population at risk during the interval. When a sheep became infected, it was excluded from the population at risk, for that particular salmonella type, in all subsequent samplings. The population at risk during the interval was measured in sheep-days, being the number of sheep at risk at the beginning of the interval multiplied by the number of days in the interval. Incidence rates were expressed in units of "new cases per hundred sheep days". Epidemic curves of salmonella excretion were drawn for the salmonella types most commonly isolated.

All experimental sheep which died during the study were necropsied using the standard procedure.

In a related study performed at a later date as part of research voyage VOY5, faecal contamination of the feedlot with salmonellae was investigated. Samples were collected daily throughout the feedlot period from the soil surface of a single feedlot paddock containing 2,000 sheep. Samples were collected wherever diarrhoeic faeces were visible on the surface of the soil. The number of samples collected each day varied according to the number of contaminated patches found, with an upper limit of twelve.

6.2.2 A Study of Salmonellosis Mortalities in the Assembly Feedlot

Epidemiologic patterns of salmonellosis mortalities in the feedlot phase were studied using four whole consignments of sheep. The first consignment was part of research voyage VOY5, but the remaining three were feedlot-only studies, titled SAL1, SAL2 and SAL3. The four were planned to be consecutive consignments through the feedlot, but one consignment which occurred between VOY5 and SAL1 was not able to be studied. The interval between consignments was also lengthened by trade disruptions which were occurring at the time; whereas Portland had been consigning
ships every 2 to 3 weeks prior to the disruptions, the interval lengthened to 4 to 6 weeks during the study.

The consignments took place in May, August, September and November 1989. All four consignments involved around 100,000 sheep and the feedlot period lasted around 10 days. All sheep which died in the feedlot were necropsied and their paddock of origin was recorded. If salmonellosis was suspected, samples of colon were cultured.

Incidence rates of crude and salmonellosis-specific mortality for the whole feedlot period were calculated for each of the 20 to 30 feedlot paddocks used each time. Paddock populations varied from day to day, so precise population-at-risk figures were derived by summing the total number of sheep-days which elapsed in each paddock during the feedlot phase. Mortality rates were then calculated by dividing the number of deaths in each paddock by the number of sheep-days at risk. The results were expressed as deaths per 10,000 sheep-days. Note that this mortality rate differs conceptually from those previously used in the thesis. Earlier examples used the initial population of sheep as the denominator since they involved a fixed experimental group. The rate used in this study is of the type called a "true" rate or "incidence density", as opposed to the "risk" rates or "cumulative incidences" used earlier (Martin et al 1987; Kleinbaum et al 1982).

The distribution of mortalities between paddocks within each consignment was compared by G-test to a theoretical random distribution (Poisson) to check for evidence of clustering typical of a contagious disease (Sokal and Rohlf 1981).

Further investigations were performed on the distribution of salmonella types involved in feedlot paddock epidemics. "Types" in this context implied species and/or phage types as applicable. All paddocks which had substantial epidemics were used ("substantial" being arbitrarily defined as those with salmonellosis-specific mortality rates over 4 per 10,000 sheep-days and from which 10 or more salmonella isolations
had been obtained). There were twelve such paddocks on VOY5, four on SAL1, and none on SAL2 or SAL3. In VOY5, 11 of the 12 epidemic paddocks were dominated by *S. Typhimurium* and one by *S. Bovis-morbificans*, therefore all isolates of these species were phage typed in their respective paddocks. In SAL1, the four paddocks were more mixed between *S. Typhimurium* and *S. Bovis-morbificans*, so both species were phage typed in each case. The stored, frozen salmonella isolates which had been collected from the four consignments were used and the typing was performed retrospectively. Type-specific mortality rates were calculated for each type in each of the paddock epidemics investigated.

6.2.3 An Examination of Long Term Trends in the Prevalence of Salmonella Types

A retrospective examination was made of the salmonella types present in a number of large collections of isolates that had been accrued over five years in the course of the research program. The collections all originated from necropsy samples taken from large cohorts of sheep which passed through the one assembly feedlot at Portland. There were seven main collections in all. The first three were from VOY1, VOY2 and VOY3, described in Chapter 4, which involved shipboard necropsies as well as those in the feedlot. The second three were from VOY5, SAL1 and SAL2 which were described earlier in this Chapter. They arose from feedlot necropsies but the typing was confined to paddocks in which high levels of salmonellosis occurred, although this included the great majority of total isolates. (SAL3 was not included here because the low incidence of salmonellosis generated only twenty isolates.) The last collection was from research voyage VOY8 whose main purpose was a field trial (described fully in Chapter 7). The VOY8 collection consisted of samples from a large cohort (22,000 sheep) taken while on board ship. The seven collections spanned a five year period from 1986 to 1990.
Records of all isolates in the collections were examined and any species testing which had not been concluded or any phage typing of *S. Typhimurium* or *S. Bovis-morbificans* which had not been performed were consequently completed. The distribution of salmonella types across all isolates, aggregated within each collection, was determined.

### 6.3 Results

#### 6.3.1 Patterns of Faecal Excretion of Salmonellae

The weather during the feedlot phase of VOY4 was extremely wet and windy, clearly sufficient to provide cold stress on the sheep.

Sixteen of the 600 experimental sheep (2.7%) died from salmonellosis during the study, nine others died of inanition (1.5%), and six of other causes (1.0%), giving a total attack rate for mortality of 5.2%. The consignment as a whole also suffered high mortality, with a whole voyage mortality rate of 4.0%.

A total of 26% of the experimental group excreted salmonellae at some time during the study period. There were five separate species isolated - *S. Typhimurium*, *S. Bovis-morbificans*, *S. Derby*, *S. Havana* and *S. Infantis*. The *S. Typhimurium* isolates included six separate phage types (9, 201a, 201, 8, 30 and 6) plus some untypable strains, and the *S. Bovis-morbificans* consisted of 3 separate phage types (10, 13 and 19) plus untypable strains. Therefore, a total of 14 separate salmonella types were isolated. The most common strains, in order of frequency, were *S. Typhimurium* PT 9, *S. Bovis-morbificans* PT 10, *S. Typhimurium* PT 201a, and *S. Havana*. Together, these four types accounted for 82% of all isolates.
No salmonellae were isolated from the 600 samples collected from sheep before leaving their farms of origin. However, within the first week of sampling, and after only six days in the assembly feedlot, excretion was widespread - 15.2% of the experimental group had begun excreting salmonellae, and thirteen of the eventual fourteen salmonella types had been isolated.

The epidemic curves for faecal excretion of the four commonly isolated salmonella types are shown in Figure 6.1. All four curves peaked at around one week after arrival in the feedlot. Also marked on the Figure is the epidemic curve of salmonellosis mortalities which followed after the excretion peaks had passed. Epidemic curves for excretion of the other salmonella types followed similar trends to those shown for the four common types, but the much lower numbers of cases made the patterns less distinct.
Figure 6.1 Epidemic curves for the incidence of faecal excretion of the four commonly isolated salmonella types. The salmonellosis mortality curve is also shown.
In the study of faecal contamination of a feedlot paddock performed as part of VOY5, fifty samples of faecally contaminated soil were collected from the paddock during the seven days of the feedlot period. The number collected per day varied from two to twelve. Salmonellae were found in 20 of the 50 samples (40%), especially from those collected later in the feedlot period (Figure 6.2).

![Graph showing salmonella isolations from faecally contaminated soil on VOY5](image)

**Figure 6.2** Salmonella isolations from faecally contaminated soil on VOY5. The no. positive and the no. of samples on each day are shown, and the graphs represent the result as a percentage.

6.3.2 Salmonellosis Mortalities in the Assembly Feedlot

The crude and salmonellosis-specific mortality rates of the four sequential consignments declined markedly from consignment to consignment during the study, particularly between SAL1 and SAL2. Crude mortality rates decreased from around
0.5% for the initial two consignments to around 0.1% for the last two. Similarly salmonellosis-specific mortality rates declined from around 0.3% to around 0.04%. The data are shown in Table 6.1.

Table 6.1 Overall feedlot mortality rates (%) for the four consignments studied

<table>
<thead>
<tr>
<th></th>
<th>Crude mortality rate</th>
<th>Salmonellosis-specific mortality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>VOY5</td>
<td>0.38</td>
<td>0.29</td>
</tr>
<tr>
<td>SAL1</td>
<td>0.53</td>
<td>0.32</td>
</tr>
<tr>
<td>SAL2</td>
<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>SAL3</td>
<td>0.11</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Salmonellosis mortality rates within paddocks ranged from 0 up to 34 deaths per 10,000 sheep-days in the VOY5 consignment, but only up to 7.5 per 10,000 sheep-days in SAL1, 3.0 per 10,000 sheep-days in SAL2, and 1.6 per 10,000 sheep-days in SAL3 (Table 6.2).

Within every consignment, the distribution of mortalities was significantly clustered (or over-dispersed) compared to a Poisson distribution (P<0.05), in the typical pattern of a contagious disease (Sokal and Rohlf 1981).
<table>
<thead>
<tr>
<th>Paddock no.</th>
<th>VOY5 May 1989</th>
<th>SAL1 August 1989</th>
<th>SAL2 September 1989</th>
<th>SAL3 November 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0 (18)</td>
<td>0.8 (2)</td>
<td>1.0 (3)</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>4.2 (11)</td>
<td>4.0 (3)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>4</td>
<td>11.4 (5)</td>
<td>1.3 (5)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>5a</td>
<td>8.3 (5)</td>
<td>0.4 (3)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>5b</td>
<td>14.8 (37)</td>
<td>*</td>
<td>*</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>6a</td>
<td>-</td>
<td>0.9 (7)</td>
<td>0.0 (0)</td>
<td>0.3 (1)</td>
</tr>
<tr>
<td>6b</td>
<td>2.0 (4)</td>
<td>1.1 (6)</td>
<td>-</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>7</td>
<td>0.0 (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>3.1 (11)</td>
<td>0.6 (7)</td>
<td>0.3 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>9b</td>
<td>-</td>
<td>0.0 (0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>11.3 (34)</td>
<td>7.5 (52)</td>
<td>0.5 (2)</td>
<td>0.2 (1)</td>
</tr>
<tr>
<td>11</td>
<td>4.0 (12)</td>
<td>6.1 (43)</td>
<td>0.5 (2)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>12a</td>
<td>34.3 (24)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12b</td>
<td>3.4 (12)</td>
<td>0.6 (2)</td>
<td>0.3 (1)</td>
<td>0.7 (3)</td>
</tr>
<tr>
<td>13a</td>
<td>6.4 (16)</td>
<td>0.1 (1)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>13b</td>
<td>2.0 (5)</td>
<td>4.9 (31)</td>
<td>0.9 (1)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>14a</td>
<td>16.4 (23)</td>
<td>-</td>
<td>-</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>14b</td>
<td>0.3 (1)</td>
<td>-</td>
<td>0.0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>1.1 (6)</td>
<td>0.3 (1)</td>
<td>0.6 (2)</td>
</tr>
<tr>
<td>16</td>
<td>5.4 (19)</td>
<td>0.0 (0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>5.1 (18)</td>
<td>2.9 (18)</td>
<td>0.0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>0.5 (2)</td>
<td>2.3 (9)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19a</td>
<td>4.6 (13)</td>
<td>1.7 (12)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>19b</td>
<td>2.5 (7)</td>
<td>0.6 (2)</td>
<td>0.0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>20a</td>
<td>6.5 (13)</td>
<td>1.9 (12)</td>
<td>0.9 (3)</td>
<td>-</td>
</tr>
<tr>
<td>20b</td>
<td>6.4 (17)</td>
<td>-</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>21</td>
<td>1.0 (2)</td>
<td>0.2 (1)</td>
<td>3.0 (12)</td>
<td>0.9 (3)</td>
</tr>
<tr>
<td>22</td>
<td>0.5 (1)</td>
<td>3.8 (30)</td>
<td>3.0 (12)</td>
<td>1.6 (7)</td>
</tr>
<tr>
<td>H2</td>
<td>0.0 (0)</td>
<td>-</td>
<td>-</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>H3</td>
<td>0.0 (0)</td>
<td>-</td>
<td>-</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>H4</td>
<td>1.2 (1)</td>
<td>-</td>
<td>0.0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>M1</td>
<td>-</td>
<td>4.6 (39)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>M2</td>
<td>-</td>
<td>3.2 (28)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>M3</td>
<td>-</td>
<td>1.1 (10)</td>
<td>0.0 (0)</td>
<td>-</td>
</tr>
</tbody>
</table>

* Dashes signify that the paddock was not in use for that consignment.
Investigation of the salmonella types involved in specific paddock epidemics showed that most of these epidemics involved a wide range of different salmonella types. *S. Typhimurium* PT 201a was the most common type of all. Figure 6.3 shows the salmonellosis mortality rates caused by each type of salmonella in the paddocks which were investigated from VOY5. Figure 6.4 shows the rates from SAL1.
Figure 6.3 Salmonellosis-specific mortality rates, by type of salmonellae involved, among twelve high salmonellosis paddocks on VOY5.
Figure 6.4 Salmonellosis-specific mortality rates, by type of salmonellae involved, among four high salmonellosis paddocks in SAL1.
6.3.3 Long Term Trends in the Prevalence of Salmonella Types

The compositions of the seven salmonella collections made from sheep passing through the assembly feedlot over five years were not similar. In the 1986-87 collections (VOY1 to VOY3) S. Derby was a common isolate, along with S. Typhimurium PT 201. In addition, S. Typhimurium PTs 202, 29 and 64 figured prominently, as well as S. Bovis-morbificans PT 07. However, by 1989-90 (VOY5, SAL1, SAL2 and VOY8) S. Typhimurium PT 201a dominated. Note that PT 201a is distinct in phage pattern from PT 201, although it would take a relatively minor genetic shift to change from one to the other (D. Lightfoot, pers. comm.). S. Typhimurium PTs 202, 64 and 29 are quite different in phage type pattern from the 201s and from each other.
### Table 6.3 Salmonella types involved in seven major collections of necropsy isolates at the Portland feedlot over five years

<table>
<thead>
<tr>
<th>Date</th>
<th>Study name</th>
<th>Size of group from which necropsies derived</th>
<th>No. of cases from which salmonellae isolated</th>
<th>No. of separate types isolated</th>
<th>Major types and the percentage of cases involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 1986</td>
<td>VOY1</td>
<td>10,060 (feedlot &amp; ship)</td>
<td>41</td>
<td>12</td>
<td>24% tm† 201</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22% derby</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22% bm† 07</td>
</tr>
<tr>
<td>January 1987</td>
<td>VOY2</td>
<td>10,504 (feedlot &amp; ship)</td>
<td>38</td>
<td>14</td>
<td>24% derby</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11% tm 201</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11% tm 29</td>
</tr>
<tr>
<td>June 1987</td>
<td>VOY3</td>
<td>10,260 (feedlot &amp; ship)</td>
<td>61</td>
<td>15</td>
<td>33% derby</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23% tm 202</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11% tm 64</td>
</tr>
<tr>
<td>May 1989</td>
<td>VOY5</td>
<td>107,120 (feedlot only)</td>
<td>243*</td>
<td>16</td>
<td>58% tm 201a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 1989</td>
<td>SAL1</td>
<td>105,524 (feedlot only)</td>
<td>163*</td>
<td>15</td>
<td>41% tm 201a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19% bm 23</td>
</tr>
<tr>
<td>September 1989</td>
<td>SAL2</td>
<td>108,067 (feedlot only)</td>
<td>37*</td>
<td>5</td>
<td>54% tm 201a</td>
</tr>
<tr>
<td>August 1990</td>
<td>VOY8</td>
<td>21,864 (ship only)</td>
<td>53</td>
<td>5</td>
<td>60% tm 201a</td>
</tr>
</tbody>
</table>

* Isolates from high salmonellosis paddocks only.
† tm = Typhimurium; bm = Bovis-morbillicans; followed by phage type number.
6.4 Discussion

The findings confirmed that salmonellosis epidemics in the live sheep trade were typical of those seen in many other intensive animal husbandry situations. From this, we could assume that the stresses of transition from farm to feedlot were the major cause of the disease. In the live sheep trade, this transition included transportation, food and water deprivation, social upheaval, cold stress (at times), and the forced adaptation to a new form and type of food - all of which occurred before the sheep get on to a ship!

The studies confirmed that salmonellosis was consistently present and widespread among sheep in the live sheep trade. Infection with a wide range of salmonella types appeared to spread rapidly through the population soon after arrival in the feedlot. The incidence of salmonella excretion among sheep was high, substantially higher than the incidence of mortality from salmonellosis. On VOY4 26% of sheep excreted salmonella at some time, yet only 2.7% died from the disease. This showed that most infections were non-fatal, a similar picture to that recently reported by Higgs et al (1993) from Western Australia and Corrier et al (1990) in calves in the USA.

The epidemic of salmonella infections also occurred before the epidemic of mortalities. Infections rose rapidly from a zero prevalence prior to entering the feedlot, to a peak around one week later, followed by a decline by the end of the second week. The consequent mortality peak lagged about a week behind the infection peak.

This study corroborated the observation in Chapter 4 (VOY1 to VOY3) that salmonellosis epidemics appear to be self-limiting in the live sheep trade. It is noteworthy that in the VOY4 study described here, where the feedlot period was extended to two weeks and where adverse weather would certainly have exacerbated stress, the salmonellosis epidemic still passed within a similar time to those observed on VOY1 to VOY3 and was not extended by the extended feedlot period. A study
under similar circumstances in cattle (Frost et al 1988) also observed this phenomenon and suggested that rumen fluid changes due to the high energy rations may inhibit salmonella. Thus the pattern may reflect an effective limitation to salmonellosis epidemics in these circumstances.

As for the method of spread of salmonella infections, the positive results from soil sampling on VOY5 confirmed that oral-faecal cycling could occur quite easily in the feedlot environment, particularly via the hay which is fed loose on the ground. However, the wide range of different salmonella types, even within specific paddock epidemics, indicates that there is more to these outbreaks than simple propagation, a fact supported by recent studies in Western Australia (Higgs et al 1993).

A key question related to the origins of the salmonella infection. The sheep may have brought infection into the feedlot each time a consignment was received; or infection may have persisted in the feedlot environment between consignments, and infected each batch anew. Of course both were likely to occur, but did one predominate? These studies provided no conclusive evidence that incoming sheep were a source, but reports from other similar situations indicated that this was extremely likely (Nottingham and Urselmann 1961; Grau et al 1968; Williams and Newell 1970; Samuel et al 1981).

On the other hand, the studies did provide indirect evidence, from a number of findings, that infection persisted in the feedlot environment between consignments.

Firstly, the consecutive feedlot studies (VOY5, SAL1, SAL2 and SAL3) showed that some salmonella types recurred consistently from consignment to consignment. Secondly, examination of the seven salmonella collections from the feedlot over five years showed that long term changes occurred in the dominant type. S. Derby was the most common type through 1986-87 (VOY1 to VOY3), and S. Typhimurium PT 201a was most common through 1989-90 (VOY5 to VOY8). Thirdly, there was a major decline in salmonellosis mortality rates between VOY5 and SAL3 which coincided with
the slow-down in feedlot throughput. The longer breaks between consignments could have allowed sun and wind to destroy salmonellae in the feedlot environment. The decrease in mortality rates was to a level unprecedented either in my previous studies or the feedlot manager’s experience, and no other changes were apparent which could explain it. Fourthly, the most recent dominant type, *S. Typhimurium* PT 201a, which occurred across most paddocks in the feedlot, was not a common phage type in sheep in this country. The National Salmonella Surveillance Scheme run by the Australian reference laboratory for *S. Typhimurium* has recorded the phage types isolated from sheep for the last five years, and among its large collection of isolates including many different phage types, PT 201a has been seen only three times outside the Portland feedlot (D. Lightfoot, pers. comm.). Similarly, a recent report from the Australian Salmonella Reference Centre, which showed *S. Typhimurium* as the major isolate from sheep, noted that PT 9 was dominant, and made no mention of PT 201a (Murray 1994). Therefore, it was unlikely that the dominance and widespread occurrence of PT 201a across the feedlot reflected the strains found in the population from which the export sheep came.

If *S. Typhimurium* PT 201a infection did arise from a reservoir within the feedlot environment, then this source was apparently a major contributor to the total rate of clinical infections and of deaths.

There are several potential locations for salmonella survival in the feedlot, including soil, water, and food. Paddock soil is probably the most likely. Faecal contamination of paddock soil with salmonellae was clearly demonstrated in the results from VOY5. A soil based reservoir would also explain the beneficial effects of the industry slow-down between VOY5 and SAL3 on salmonellosis rates. Salmonellae can survive quite well in external environments provided they are protected from direct sunlight and desiccation (Robinson et al 1970; Robinson and Royal 1971; Wray and Sojka 1977). In practice, the feedlot paddocks are usually covered with a deep (2 to

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*Microbiological Diagnostic Unit, University of Melbourne, Parkville, Vic, Australia*
10 cm) humus layer comprising crushed hay, sheep manure and sand. Pools of ground water also occur on the feedlot, especially in winter, and these seem to be favoured as drinking sites by many sheep, even if they are small, muddy puddles. Given the importance of salmonellosis in the live sheep trade, it seems imperative now to further investigate the existence of feedlot reservoirs of salmonella infection.

6.5 Summary

Salmonellosis in the live sheep trade occurred immediately following the transportation and mixing of sheep in the assembly feedlot environment. Salmonella excretion increased rapidly soon after the arrival of sheep in the feedlot, not just from a single propagating infection, but from many different types of salmonellae simultaneously. Some of the infected sheep died, creating a mortality peak which followed about one week after the excretion peak. The epidemic appeared to pass of its own accord, indicating that salmonellosis outbreaks in the live sheep trade were self-limiting.

The studies also presented evidence that salmonella infection persisted in the feedlot environment between consignments. The evidence included: the consistent recurrence of certain salmonella types in the feedlot, including some which are uncommon in the general sheep population; the long term trends showing changes in the dominant types found in the feedlot; and the observed decline in salmonellosis that occurred following a slow-down in feedlot use. None of these were definitive, but collectively they suggested that salmonella reservoirs did exist.

New recommendations for the control of salmonellosis in the live sheep trade could be made as a result of these findings. It was recommended that the industry introduce measures which prevent the propagation of salmonellosis, by feeding hay in hay racks rather than on the ground, and by preventing sheep from drinking ground
water. Also, research should be commissioned to investigate the existence of salmonella reservoirs in the feedlot environment.
CHAPTER 7

A STUDY OF THE SPATIAL PATTERNS OF SHEEP MORTALITY ON BOARD SHIPS

7.1 Introduction

In the observational studies on VOY1 to VOY3 (Chapter 4) an unexpected finding was made that mortality rates differed depending upon where sheep were housed on board ship. Neither the review of the literature (Chapter 1) or the analysis of Masters' Reports (Chapter 3) had suggested that this might occur. However, the magnitude of the effects were so great that further investigation was warranted.

The major effect was that sheep in upper tier pens had higher mortality rates than sheep in lower tier pens. Few physical differences between the tiers were apparent, and none had been considered likely to cause health effects. Upper tier pens were lighter than lower tier pens because they were closer to ceiling lights. For the researchers, this made the upper tier environment more pleasant rather than less because it enabled them to see and be seen more easily by the sheep when they entered the pens, and thus avoid the jostling which they received in the darker, lower tier pens! Another difference of a more theoretical kind related to the "visual cliff" which surrounded upper tier pens. Sheep in the upper tier pens faced a drop of about 1.5 metres on all sides through the barred walls of the pen. They also had to face this cliff directly when eating or drinking because the food and water troughs were mounted on the outside of the bars of the pen wall overlooking the corridors (see Figure 1.3). However, no evidence or suggestion had previously come forth that these effects would cause differences in mortality rates.
Experiments were undertaken to confirm and then investigate the "tier effect". They involved, as a first step, repeating the observations initially made on VOY2 and VOY3 and analysing the data more completely. A study was then performed to catalogue and measure the differences which existed between tiers and rows, both in terms of sheep behaviour and the physical characteristics of the pens. Finally, a field trial was performed to test the hypothesis that light intensity caused the tier effect.

7.2 Materials and Methods

7.2.1 Additional Observations of the Spatial Patterns of Mortality On Board Ship

Two research voyages, VOY5 and VOY6, were used to gather further census data on the location of sheep deaths on board ship. Similar methods to VOY2 and VOY3 were used (Chapter 4) and analyses were performed as described in Section 2.2. On VOY6 the consignment included a large group of "young" wethers (28,000 3-year olds) so that two separate analyses for that voyage were performed - one for "adult" wethers and one for "young" wethers. In addition, separate mortality rates for the first, middle and last thirds of each voyage were calculated and analysed in order to explore any temporal changes in the patterns observed.

7.2.2 Observations on Sheep Behaviour and Environmental Differences Between Tiers and Rows

A study was designed for VOY5 to investigate a range of factors potentially associated with the tier and row effects. The study design had aspects of both a cohort observational study and a designed experiment. It involved the measurement of a variety
of aspects of sheep behaviour and pen environment from a set of shipboard pens chosen to cover different tier and row combinations. The set included upper and lower tier pens in each of three rows (the outer, intermediate, and inner, port-side rows) replicated three times on adjacent decks. Pens were the experimental units, and the study was considered as a 2 x 3 factorial design in 3 randomised blocks, where decks represented the blocks. A total of 18 pens was therefore involved. Port versus starboard effects were not addressed.

The pens referred to here were actually sub-divisions of the larger "pens" used in the census studies. In this study the internal gates within the larger pens were closed, creating smaller areas with a capacity of around 100 sheep (as described in Section 1.1). The pens were chosen to be near the aft end of the ship for the purpose of access and handling of the sheep. However, the aft-most pens were avoided because they were considered likely to be influenced by their outside, weather-exposed position. The pens chosen were the second pens in from the aft end, which is illustrated schematically in Figure 7.1.

The 18 pens were each filled with 100 sheep, comprising 10 sheep from each of 10 truck groups. This approach was taken to even out any effects of source of sheep on pens. The sheep had been selected and ear-tagged on arrival at the assembly feedlot a week earlier. Trucks had been selected by convenience and 200 sheep were taken from each (this gave extra sheep to allow for losses prior to the loading of the ship). The whole group had been run together in one feedlot pen throughout the feedlot period. Practical difficulties during loading thwarted the complete random allocation of 10 sheep from each source to each pen, and some later rearrangement of sheep had to be completed after loading. This took place within the first three days of the voyage, and involved a total of 42 sheep.

All sheep which died from the experimental group were necropsied using the standard procedure. The pathologist was not informed as to the pen of origin of the dead sheep at necropsy. Crude and cause-specific mortality rates (for INANITION,
**Figure 7.1** Schematic plan of the experimental pens used at the aft end of the sheep house for observations on sheep behaviour and environmental differences between tiers and rows.

SAL/ENT and OTHER CAUSES) were derived for each pen.

All experimental sheep were weighed one day prior to sailing and again at the end of the voyage, to determine the average weight change for each pen.

Feeding behaviour was measured both before and during the voyage using the food dye technique (see 2.3.2 for details). The method was used to indicate pellet consumption for each sheep on six occasions; the first two were at Days 3 and 6 post-arrival in the feedlot, and the next four were on board ship at four day intervals. The use of dyed food on board ship had to be staggered to allow for the workload, with one block (deck) being measured each day in a four day rotation. The results were used for two purposes. Firstly, "persistent non-feeders" were identified, as previously defined in Chapter 5 (5.2.3). Such animals were assumed to be suffering from the inanition
syndrome. The count of these cases, plus all inanition deaths, was used as a measure of the total morbidity from inanition, as opposed to mortality, for each pen. The second use of food dye results was to measure the conversion of sheep from "non-feeders" to "feeders" while on board ship. The number of sheep which were non-feeders at the point of sailing, as defined by two negative feed dye readings in the feedlot, was used as a baseline. The percentage of that initial group which failed to convert at each of the subsequent shipboard dye readings was calculated for each pen.

The rate of food consumption was used as a measure of group feeding behaviour. On four occasions, at four day intervals throughout the voyage, the rate of disappearance of food from the food troughs over the first four hours post-feeding was measured volumetrically. By the fourth round of measurements, late in the voyage, virtually all feed was consumed within the four hour period, so this last set of data was not used in analysis. Instead the mean of the first three rounds was used for each pen.

Behavioural responses to stimuli were also measured at four day intervals on each experimental pen. The tests were performed by the same person each time (me) wearing the same clothing (khaki overalls). In the first test, the number of sheep feeding at a trough was counted, then the group was quietly approached and a hand was waved briefly in front of them. The number of the sheep which withdrew their heads, and the number which returned within 30 seconds was recorded. In the second test, a piece of stiff, black polythene pipe was rattled firmly between the bars of the pen wall for five seconds, and the radius of the clear zone of withdrawal of the sheep was measured with a measuring stick. The tests were done in random order on the pens in one block (deck) each day in a four day rotation. The "human approach" test was performed on all pens first, followed later by the "rattling stick" test. Because the tests involved the presence of a person in the corridor, which could influence more than one experimental pen, at least five minutes were allowed to elapse between tests in any one corridor. The behavioural tests gave four data for each pen. Two of them - the number of sheep present and percentage which returned within 30 seconds of my approach - were considered to indicate motivation to come forward and feed. The other two - the
percentage which withdrew from human approach and the radius of withdrawal from the sudden noise - were considered to indicate fearfulness. The mean of each of these variables for each pen over all four rounds of measurement was used in the analysis.

Environmental measurements involved temperature, humidity and light intensity. The first two were measured on each pen three times per day, at 7 am, 3 pm and 11 pm, throughout the voyage. Light intensity was only measured at 7 am and 3 pm because readings after dark were usually zero. Temperature and humidity were measured in the approximate centre of each pen with an electronic meter attached to a three metre pole. Temperature was measured to the nearest 0.1°C and relative humidity to the nearest unit of %. Light intensity was measured in the centre of each pen, and over each food and water trough. An electronic lux meter was used which measured to the nearest unit of lux. Each of the three times of day were analysed separately for these variables. The datum used for each pen was the mean, over all days of the voyage, of log_{10}(light intensity + 1). This transformation was used because of the skewed distribution of the data and occasional zero readings.

The difference between tiers in cause-specific mortality and total morbidity from inanition were statistically assessed using Wilcoxon's signed rank test (Sokal and Rohlf 1981), using the two pens in each row x deck combination as a pair. This non-parametric test was used for mortality and morbidity because the data were too sparse for parametric analysis. The test was used as a one-tailed test on the basis that incidence rates were expected to be higher in upper tiers.

The feeding, behavioural and environmental data for each pen were examined for their associations with row and tier. Note that the design was intended to examine associations of these environmental and other factors with location, rather than directly with mortality or morbidity. The earlier and larger census study (7.2.1) had established the patterns of mortality, this study was to examine other factors which may be explanatory. The actual mortality rates during the study were not directly relevant and were only measured to check that patterns consistent with expectation occurred.
Associations were examined using analysis of variance (ANOVA). Initially, the ANOVA model used was to have included TIER and ROW main effects plus an interaction term. Deck differences were to have been analysed as experimental blocks. However, when deck differences did occur unexpectedly in some data, the model was changed to include DECK as well as TIER and ROW main effects, and all two-way interactions. The three way interaction was used as the error term since in this analysis there was no replication. The results reported are those of the latter model.

After VOY5 was completed, the observations on feeding behaviour differences between tiers were extended on a subsequent voyage, VOY7. Only brief observations were possible because of other work commitments. On each day after Day 3 of the voyage, the number of sheep feeding at food troughs was counted in twelve pairs of upper and lower tier pens containing adult wethers. The observations were always made within one to two hours of feeding. Observations were made from the aft end of the sheep house, by looking down the alley-ways and counting the number of sheep present at the first two food troughs in upper and lower tier pens (effectively counting about five metres down the alley). The sheep were not disturbed by the observer. The counts for each pen were summed over the whole voyage as a composite measure of feeding intensity. The data were analysed by an analysis of variance similar to that used for the spatial distribution analyses described in Section 7.1. Three factors were included, describing each pen in terms of DECK, TIER and ALLEY. ALLEY was used here instead of SIDE and CENTRALNESS because the food troughs of two adjacent rows were present in the same alley. One alley served Rows A and B, another served Rows C and D, and a third served Rows E and F. Because of this layout, ALLEY seemed a more relevant descriptor. The full three factor factorial model was used. Analyses were also performed on the numbers feeding over the first, middle and last thirds of the voyage.
7.2.3 A Field Trial of Reduced Light Intensity as a Treatment for the Tier Effect

A field trial involving the reduction of light intensity in upper tier pens was performed on research voyage VOY8. Only two treatments were involved - reduced light intensity and normal light intensity, and only upper tier pens were used. In order to reduce light intensity in a pen, all the sheep house lights surrounding the pen were shaded with black steel light shades. The shades curved around one side of the fluorescent lights, preventing light from shining into the adjacent pen. The design is illustrated in Figure 7.2.

The experimental units were shipboard pens. The shipment carried five decks of adult wethers, and all decks were used in the trial. However, to avoid the complication of natural sunlight affecting light intensity, none of the outer parts of the sheep house were used. This included the entire port and starboard rows (A and F) and even the fore- and aft-most parts in the other rows (B to E). The latter areas were excluded by closing the end-most subdivisional gate within each pen, so separating the last 9 metres, and approximately 100 sheep, from the rest of the pen. Thus each deck contributed 12 pens, four rows wide by three sections long, and a total of 60 pens were available for the trial. The number of sheep in each pen was obtained from the ship’s loading plan. In total the experiment involved 21,834 sheep.

The experiment was a balanced, extended block design, with blocking used to minimise the variation in mortality rates. From prior experience with spatial patterns (Chapter 4) it was assumed that there would be little variation fore-to-aft along the sheep house, but a larger amount of side-to-side variation. For this reason, the fore, middle and aft sections of two adjacent rows were used as an experimental block. One of the two rows in each block was chosen at random to receive one light and two dark pens,

*Where the end-most sections of rows B to E had been closed off, the number of sheep in the excluded area was counted and subtracted from the plan figure to give the number remaining in the experimental part of the pen.
Figure 7.2 Horizontal views of lights in an alley on board ship. Diagram shows how light shades were used to reduce light intensity in upper tier pens. Photograph shows one shade in place.
and the other received two light and one dark. The allocation of treatments within rows was also random, within these constraints. Figure 7.3 is a floor plan showing an example allocation for one deck.

All sheep which died were identified as to their pen of origin by the ship’s crew, using numbered plastic tags which were placed at every one of the experimental pens. The implementation of the system was accompanied by intensive briefings and close supervision so as to avoid the problems with poor identification of sheep, which were encountered on VOY3. Standard necropsies were performed on all dead sheep by a pathologist not informed as to which treatment any sheep had received. The causes of death were categorised into INANITION, SAL/ENT and OTHER CAUSES.

Attack rates of mortality, both crude and cause-specific, were calculated for each pen by dividing the number of sheep which died, by the number originally present in the pen. Rates were calculated for the whole voyage as well as for the first, middle and last thirds separately.

Temperature, humidity and light intensity were measured in the centre of each experimental pen each day. Light intensity was also measured daily over three food troughs in each section. The lights on all pens were checked daily and any dislodged shades or malfunctioning lights were fixed immediately.

The differences between light and dark pens were analysed by analysis of variance, taking block and row variations into account by including variables for BLOCK, and ROW-within-BLOCK. Mortality rates were subject to angular transformation, and back-transformed means were bias-corrected using the first order Taylor’s approximation previously described (Section 2.2). The light intensity data used were the means of $\log_{10}(\text{lux} + 1)$. 

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Figure 7.3 Floor plan of the experimental layout within a deck. Alternate rows received complimentary light/dark ratios, but within a row, the choice of which pen received which was random.
7.3 Results

7.3.1 Spatial Patterns of Mortality On Board Ship

The crude mortality rates for the four voyages analysed for spatial distribution ranged generally around 1% to 2%. The results are shown in Table 7.1.

<table>
<thead>
<tr>
<th>Voyage</th>
<th>No. of sheep</th>
<th>No. which died during voyage</th>
<th>Crude mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VOY2</td>
<td>69,150</td>
<td>729</td>
<td>1.05</td>
</tr>
<tr>
<td>VOY3</td>
<td>89,650</td>
<td>1,026</td>
<td>1.14</td>
</tr>
<tr>
<td>VOY5</td>
<td>54,107</td>
<td>1,191</td>
<td>2.20</td>
</tr>
<tr>
<td>VOY6</td>
<td>42,097</td>
<td>469</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>(adult wethers)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOY6</td>
<td>28,461</td>
<td>261</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>(young wethers)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In each of the five separate analyses, upper tier pens had substantially and significantly higher mortality rates than lower tier pens. There were also significant differences, in four of the five analyses, between rows across the ship; that is, either SIDE or CENTRALNESS or both were significantly associated with mortality.
However, the side-to-side pattern of mortality rates varied between voyages. Significant higher order interactions occasionally occurred, but not in a consistent pattern. It was assumed that they arose from inconsistent differences between pens of sheep. The significance levels of the main effects of all five location factors in the five separate analyses are presented in Table 7.2.

<table>
<thead>
<tr>
<th>Voyage</th>
<th>DECK</th>
<th>TIER</th>
<th>SECTION</th>
<th>SIDE</th>
<th>CENTRALNESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>VOY2</td>
<td>not signif.*</td>
<td>P&lt;0.001</td>
<td>not signif.</td>
<td>P&lt;0.025</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>VOY3</td>
<td>not signif.</td>
<td>P&lt;0.001</td>
<td>not signif.</td>
<td>P&lt;0.005</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>VOY5</td>
<td>not signif.</td>
<td>P&lt;0.001</td>
<td>not signif.</td>
<td>P&lt;0.001</td>
<td>not signif.</td>
</tr>
<tr>
<td>VOY6</td>
<td>not signif.</td>
<td>P&lt;0.001</td>
<td>not signif.</td>
<td>not signif.</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>VOY6 (adults)</td>
<td>not signif.</td>
<td>P&lt;0.025</td>
<td>not signif.</td>
<td>not signif.</td>
<td>not signif.</td>
</tr>
<tr>
<td>VOY6 (young)</td>
<td>not signif.</td>
<td>P&lt;0.025</td>
<td>not signif.</td>
<td>not signif.</td>
<td>not signif.</td>
</tr>
</tbody>
</table>

* Not significant implies P>0.05.

Upper tier mortality rates were consistently between 1.5 and 2.4 times higher than lower tier mortality rates in all five analyses. These figures would be analogous to relative risks for mortality in upper versus lower tiers. The results are shown graphically in Figure 7.4.

The side-to-side differences in mortality rate varied from voyage to voyage. VOY2, VOY3 and VOY6 (adults) showed a tendency for lower mortality rates in rows
near the mid-line of the ship, although the details of their patterns were different. VOY5 mortalities declined from left to right, and VOY6 (young) showed a trend, although not significant, for declining mortalities from right to left. The side-to-side variations are shown in five separate graphs in Figure 7.5.

Separate analyses of the first, middle and last thirds of the voyages simply showed that both the tier effect and the side-to-side patterns occurred consistently throughout each voyage.
Figure 7.5 Bias-corrected, back-transformed mortality rates across the ship, as described by the location factors SIDE and CENTRALNESS.
7.3.2 Sheep Behaviour and Environmental Differences Between Tiers and Rows

The crude mortality rate for the 1,800 experimental sheep involved with the VOY5 study was 1.9%, of which 0.8% was attributed to INANITION, 0.6% to SAL/ENT, and 0.5% to OTHER CAUSES. One of the ten source groups had particularly severe mortality problems, accounting for half of all deaths in the experimental group. Severe weight loss, as previously defined by the loss of 15% or more of body weight during the voyage, affected 5.2% of the experimental group. There were 3.2% classified as "persistent non-feeders".

The total number of experimental sheep which died in upper tier pens was significantly greater than the number in lower tiers. Similarly, the number of INANITION deaths, and the number of SAL/ENT and OTHER CAUSES deaths combined, were significantly greater in upper than lower tier pens. (There were too few SAL/ENT and OTHER CAUSES deaths to give significant differences when considered separately, although trends for both were in the same direction.) However, the morbidity from INANITION, being INANITION deaths plus "persistent non-feeders", was not significantly different between tiers. The median and range of the numbers per pen affected with each of these health outcomes in upper versus lower tier pens, is presented in Table 7.3.

The number of severe weight loss cases did not differ between tiers, as shown in Table 7.4.
Table 7.3  The median (and range) of numbers of sheep affected per pen by four health outcomes, in upper and lower tier experimental pens on VOY5

<table>
<thead>
<tr>
<th></th>
<th>Total deaths</th>
<th>INANITION deaths</th>
<th>Deaths from causes other than INANITION (SAL/ENT + OTHER CAUSES)</th>
<th>Morbidity from INANITION (deaths + persistent non-feeders)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>3 (0-7)</td>
<td>1 (0-4)</td>
<td>2 (0-3)</td>
<td>4 (0-10)</td>
</tr>
<tr>
<td>Lower tier</td>
<td>1 (0-3)</td>
<td>0 (0-2)</td>
<td>0 (0-3)</td>
<td>3 (1-4)</td>
</tr>
<tr>
<td>Significance of difference*</td>
<td>P=0.016</td>
<td>P=0.031</td>
<td>P=0.039</td>
<td>P&gt;&gt;0.05, precise value not calculated</td>
</tr>
</tbody>
</table>

* Assessed by Wilcoxon’s signed rank test, one-tailed.

Table 7.4  Numbers of severe weight loss cases (sheep which lost over 15%, 20% or 25% of their pre-embarkation body weight during the voyage) in upper versus lower tiers

<table>
<thead>
<tr>
<th></th>
<th>No. of sheep with both weights recorded</th>
<th>No. (and %) which lost over 15% of pre-embarkation weight</th>
<th>No. (and %) which lost over 20% of pre-embarkation weight</th>
<th>No. (and %) which lost over 25% of pre-embarkation weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>862</td>
<td>44 (5.1)</td>
<td>23 (2.3)</td>
<td>7 (0.8)</td>
</tr>
<tr>
<td>Lower tier</td>
<td>872*</td>
<td>47 (5.4)</td>
<td>26 (3.0)</td>
<td>8 (0.8)</td>
</tr>
</tbody>
</table>

* Total does not equal initial total (1800) due to deaths and some loss to follow-up.
The percentage of sheep which failed to convert from "non-feeders" to "feeders" was consistently higher in upper tier pens than lower tier pens. This implied that upper tier sheep were slower to adapt to feeding than those in lower tiers. At the first round of shipboard dye readings, the difference was significant (P=0.031). However, as the voyage progressed and most sheep converted, the measurement tended to approach a threshold (low) and the significance of the difference diminished (Table 7.5).
Table 7.5 Percentage of sheep which failed to convert from "non-feeders" to "feeders" in each round of the shipboard dye readings, by tiers and rows

<table>
<thead>
<tr>
<th>First shipboard dye reading</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>25</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>Lower tier</td>
<td>12</td>
<td>9</td>
<td>18</td>
</tr>
</tbody>
</table>

  Tier effect significant (P=0.031).
  Row effect not significant (P=0.312).

<table>
<thead>
<tr>
<th>Second shipboard dye reading</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>17</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Lower tier</td>
<td>9</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

  Tier effect not significant (P=0.075).
  Row effect not significant (P=0.788).

<table>
<thead>
<tr>
<th>Third shipboard dye reading</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>13</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Lower tier</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

  Tier effect not significant (P=0.122).
  Row effect not significant (P=0.918).

<table>
<thead>
<tr>
<th>Fourth shipboard dye reading</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>10</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Lower tier</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

  Tier effect not significant (P=0.395).
  Row effect not significant (P=0.572).
The measurement of food consumption proved difficult. The process relied on research staff timing the arrival of feed down the ship’s delivery chutes in order to return exactly four hours later to measure the amount that had disappeared. However, at times the food arrived too quickly and some pens on Decks 5 and 6 had access to food for a short but unknown period before timing began. When the data were analysed, significant deck to deck differences were observed that were consistent with this error. That is, Decks 5 and 6 appeared to have slower food consumption than Deck 4. However, differences between tiers were also observed which showed that upper tier pens ate significantly more slowly than lower tier pens (Table 7.6).

| Table 7.6 Volume of feed consumed (litres) over the first four hours after feeding, averaged over the first three measurements during the voyage, by tiers and rows |
|--------------------------------------------------|-------------|-------------|-------------|
| Row A    | Row B    | Row C    |
| Upper tier | 70    | 61    | 66    |
| Lower tier | 82    | 75    | 70    |

Tier effect significant (P=0.019).
Row effect not significant (P=0.118).
Deck effect also significant, not shown (P=0.002).

The behavioural data showed no clear cut effects. Only one of the four behavioural measurements showed significant differences between tiers or rows (Table 7.7). However, some trends may be seen in the data, for example, the percentage of sheep which returned to feeding within 30 seconds of human approach varied in a pattern which mirrored mortality rate patterns.
Table 7.7 Behavioural measurements, averaged over four observations made during the voyage, by tier and row

<table>
<thead>
<tr>
<th>Number of sheep initially present at food trough</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>4.2</td>
<td>3.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Lower tier</td>
<td>4.7</td>
<td>4.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Tier effect not significant (P=0.382).
Row effect not significant (P=0.415).

<table>
<thead>
<tr>
<th>Percentage retreating from human presence</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>79</td>
<td>85</td>
<td>87</td>
</tr>
<tr>
<td>Lower tier</td>
<td>80</td>
<td>93</td>
<td>86</td>
</tr>
</tbody>
</table>

Tier effect not significant (P=0.402).
Row effect not significant (P=0.138).

<table>
<thead>
<tr>
<th>Percentage of sheep returning within 30 seconds</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>45</td>
<td>37</td>
<td>33</td>
</tr>
<tr>
<td>Lower tier</td>
<td>71</td>
<td>61</td>
<td>37</td>
</tr>
</tbody>
</table>

Tier effect not significant (P=0.209).
Row effect not significant (P=0.366).

<table>
<thead>
<tr>
<th>Radius of clear zone of retreat from sudden noise (m)</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>0.92</td>
<td>0.93</td>
<td>1.08</td>
</tr>
<tr>
<td>Lower tier</td>
<td>0.84</td>
<td>0.93</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Tier effect not significant (P=0.094).
Row effect significant (P=0.036).
The environmental variables showed many significant effects, between tiers, rows and decks. These measurements apparently had much more precision than the other variables, and as a result the estimates of error used in analyses were quite small. Light intensity had the strongest association with both tier and row, with upper tiers and outer rows having higher light intensity than lower tiers and inner rows, regardless of the time of day. Table 7.8 presents the results from the 3 pm data only, since the results from the 7 am data were quite similar. There were also significant interactions between deck and tier, and row and tier, which reflected real, but small, variations in light intensity. The deck x tier interaction occurred because Deck 6 had slightly higher ceilings than other decks which led to slightly higher light intensity in upper tiers. The tier x row interaction was caused by the effect of natural light on outer rows which reduced the difference between tiers compared to that seen in more inner rows.
<table>
<thead>
<tr>
<th></th>
<th>Centre of pen</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>1.53</td>
<td>1.63</td>
<td>1.42</td>
<td></td>
</tr>
<tr>
<td>Lower tier</td>
<td>1.41</td>
<td>0.81</td>
<td>0.41</td>
<td></td>
</tr>
</tbody>
</table>

Tier effect significant (P<0.001).
Row effect significant (P<0.001).
Two interactions significant; Deck x Tier (P=0.009), Tier x Row (P<0.001).

<table>
<thead>
<tr>
<th></th>
<th>Over food troughs</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>1.95</td>
<td>1.95</td>
<td>1.91</td>
<td></td>
</tr>
<tr>
<td>Lower tier</td>
<td>1.69</td>
<td>1.69</td>
<td>1.64</td>
<td></td>
</tr>
</tbody>
</table>

Tier effect significant (P<0.001).
Row effect significant (P<0.001).
Deck effect significant, not shown (P<0.001).
Two interactions significant; Deck x Row (P=0.014), Tier x Row (P=0.024).

<table>
<thead>
<tr>
<th></th>
<th>Over water troughs</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>3.57</td>
<td>2.00</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>Lower tier</td>
<td>3.83</td>
<td>1.64</td>
<td>1.64</td>
<td></td>
</tr>
</tbody>
</table>

Tier effect significant (P<0.001).
Row effect significant (P<0.001).
Deck effect significant, not shown (P=0.021).
One interaction significant; Tier x Row (P<0.001).
The results of temperature measurements showed that outer rows were about half a degree cooler than inner rows, but tier differences were smaller still and less significant (Table 7.9). The patterns were similar at all three times of the day. Again significant deck effects occurred although the differences were less than half a degree. The pattern suggested that higher decks were cooler which was an unexpected and unexplained finding. Humidity differences were neither significant or related in any consistent way to mortality patterns.

Table 7.9 Temperatures (°C) at three times of day, by tiers and rows

<table>
<thead>
<tr>
<th>Time</th>
<th>Tier</th>
<th>Row A</th>
<th>Row B</th>
<th>Row C</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 am</td>
<td>Upper tier</td>
<td>23.54</td>
<td>23.64</td>
<td>24.11</td>
</tr>
<tr>
<td></td>
<td>Lower tier</td>
<td>23.68</td>
<td>23.71</td>
<td>24.21</td>
</tr>
</tbody>
</table>
|        |            | Tier effect not significant (P=0.088).  
|        |            | Row effect significant (P=0.001).  
|        |            | Deck effect significant, not shown (P=0.006).  |
| 3 pm   | Upper tier | 24.74  | 24.77  | 24.99  |
|        | Lower tier | 24.86  | 24.84  | 24.97  |
|        |            | Tier effect not significant (P=0.188).  
|        |            | Row effect significant (P=0.017).  
|        |            | Deck effect significant, not shown (P=0.005).  |
| 11 pm  | Upper tier | 24.60  | 24.64  | 24.84  |
|        | Lower tier | 24.74  | 24.74  | 24.90  |
|        |            | Tier effect significant (P=0.023).  
|        |            | Row effect significant (P=0.006).  
|        |            | Deck effect also significant, not shown (P<0.001).  |
The observations from VOY7 of the number of sheep present at the food troughs corroborated the findings on VOY5 regarding feeding motivation. There were significantly less sheep present at the food troughs in upper tier pens than in lower tier pens over the whole voyage in VOY7. This trend was apparent in each separate third of the voyage, although the difference was not statistically significant each time due to the smaller numbers involved. Neither of the other location factors (DECK or ALLEY) or any interaction terms were significant. The results are presented in Table 7.10. As can be seen from the Table, the numbers of sheep present at the food troughs in both upper and lower tiers increased steadily throughout the voyage. This was as expected as the sheep adapted to shipboard feeding.

<table>
<thead>
<tr>
<th></th>
<th>First third (days 3-7)</th>
<th>Middle third (days 8-12)</th>
<th>Last third (days 13-17)</th>
<th>Whole voyage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper tier</td>
<td>15</td>
<td>16</td>
<td>34</td>
<td>64*</td>
</tr>
<tr>
<td>Lower tier</td>
<td>19</td>
<td>25</td>
<td>41</td>
<td>85</td>
</tr>
<tr>
<td>Significance of difference†</td>
<td>P=0.131</td>
<td>P=0.012</td>
<td>P=0.115</td>
<td>P=0.018</td>
</tr>
</tbody>
</table>

* Figures may not sum exactly due to rounding.
† As determined by analysis of variance.
7.3.3 Reduced Light Intensity as a Treatment for the Tier Effect

The crude mortality rate of the whole consignment on VOY8 was 1.1%. This was matched by the crude mortality rate in the experimental group which also recorded 1.1%.

No significant differences occurred in crude or cause-specific mortality rates between darkened and normally lit pens. The results are presented in Table 7.11. Separate analyses of the first, middle and last thirds of the voyage showed a similar lack of significant differences.

<table>
<thead>
<tr>
<th></th>
<th>Normally lit pens</th>
<th>Darkened pens</th>
<th>Significance of difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRUDE MORTALITY</td>
<td>0.99</td>
<td>1.12</td>
<td>P=0.536</td>
</tr>
<tr>
<td>INANITION</td>
<td>0.52</td>
<td>0.38</td>
<td>P=0.180</td>
</tr>
<tr>
<td>SAL/ENT</td>
<td>0.20</td>
<td>0.34</td>
<td>P=0.060</td>
</tr>
<tr>
<td>OTHER CAUSES</td>
<td>0.27</td>
<td>0.37</td>
<td>P=0.231</td>
</tr>
</tbody>
</table>

* Assessed by analysis of variance, including BLOCK, and ROW-within-BLOCK terms.

The light shades had been quite effective in reducing light intensity in the experimental pens as shown by measurements of light intensity. The centre of the darkened upper tier pens had an average light intensity (back-transformed from the log-scale used in analysis) of 0.8 lux, compared to normally lit pens which averaged 6.4 lux. Light over the food troughs averaged 5.2 lux in dark pens and 19.9 lux in light pens.
There were no significant differences in either temperature or humidity between light and dark pens. The results of the analyses are given in Table 7.12.

<table>
<thead>
<tr>
<th></th>
<th>Normally lit pens</th>
<th>Darkened pens</th>
<th>Standard error of difference</th>
<th>Significance of difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>22.28</td>
<td>22.25</td>
<td>0.059</td>
<td>P=0.557</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td>72.2</td>
<td>71.8</td>
<td>0.37</td>
<td>P=0.373</td>
</tr>
<tr>
<td>Log_{10}(centre of pen light intensity_{lux} + 1)</td>
<td>0.87</td>
<td>0.25</td>
<td>0.033</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Log_{10}(feed trough light intensity_{lux} + 1)</td>
<td>1.32</td>
<td>0.79</td>
<td>0.070</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

* Assessed by analysis of variance, including BLOCK, and ROW within BLOCK terms.

### 7.4 Discussion

These studies have proved the existence of a "tier effect" on mortality. Sheep in upper tiers on board ship had crude mortality rates approximately twice as high as those in lower tiers in each of the five data sets that were obtained. These data sets involved four voyages, two different ships and two different types of sheep. The finding has important implications for the live sheep trade because it implies that somewhere among the subtle and uncontrived differences between tiers lies a factor or factors which substantially affects death rate.
The influence of the tier effect on specific causes of death was not clear. While preliminary observations in VOY3 indicated that inanition deaths occurred more commonly in upper than lower tiers (Table 4.11), the more specific measurements reported in the VOY5 designed observational study indicated that all causes of death differed. However, the *morbidity* rate of inanition was not different between tiers, even though the mortality rate was. This implied that the case fatality rate for sheep suffering from inanition in upper tiers was higher than the rate for sheep in lower tiers.

The VOY5 study showed that there was a diminution of the sheep’s motivation to eat in upper tiers compared to lower tiers. There were less sheep present at the food trough in upper tiers, the food was consumed at a slower rate in upper tiers, and non-feeders were slower to convert to feeders in upper tiers, when compared to their counterparts a meter and a half below. Behavioural responses, while not statistically significant, also showed a tendency for upper tier sheep to be slower to return to feeding after disturbance than lower tier sheep. These findings clearly indicated the existence of a general adverse influence on feeding in the population in upper tiers, which would explain the higher case fatality rate for sheep with the inanition syndrome. A recent study of feeding behaviour of sheep on ships, performed for another reason, made a confirming observation that sheep on upper tiers consistently ate less than those on lower tiers (Bailey and Fortune 1992).

Light intensity was proven to be higher in upper tier than lower tier pens. It was hypothesised that light might be a necessary cause (Martin *et al* 1987) of the tier effect, presumably by increasing stress in the sheep in upper tiers and/or inhibiting them from coming to the food troughs, where the light was brightest. However, the major field trial which was undertaken showed no effect on mortality when the light intensity in upper tier pens was reduced. The trial involved a large number of experimental pens, the treatment effectively reduced light intensity to levels similar to those seen in lower tier pens, and the results showed no trend for mortality rates to be reduced. Therefore, the hypothesis appears to have been effectively tested, and rejected.
There are other possible causes of the tier effect which have yet to be investigated. Noxious gases may be involved. If such gases formed vertical gradients within the air space of a deck, then upper tier pens may be more affected than lower tiers. However, there is little precedent and no observations which support this theory.

One remaining theory involves the "visual cliff". Sheep in upper tier pens are confronted by a perceived "cliff" - a 1.5 metre drop on all sides. To feed and drink, sheep must face this drop directly. Sheep have notoriously poor depth perception, thus their habit of jumping over shadows (Grandin 1980). They were also seen to be extremely fearful of going near the ship-side railing during loading or unloading. Fearfulness of the visual cliff could explain the adverse influences on feeding which were observed.

Other associations between mortality and location were seen in these studies, arising from side-to-side differences across the ship. These effects are probably just as real as the tier effect. However, the variability in the side-to-side patterns implies that the factor is not a fixed feature of the ship's construction. Ventilation is one possible factor because seasonal variations occur in wind patterns during the voyages. The variability of the effect makes the phenomenon less easy to study and less likely to be of practical value in reducing shipboard mortalities.

7.5 Summary

The major finding of the specific studies in this Chapter has been to confirm that mortalities were affected by where sheep were housed on board ship. The strongest influence was the tier effect, where mortality rates were higher in sheep in upper tier pens than those in lower tier pens, and where feeding behaviour was generally compromised in upper tiers compared to lower tiers.
One major hypothesis as to the cause of the tier effect - light intensity - was investigated in a field trial and rejected. However, a remaining hypothesis regarding the adverse effect of the visual cliff which constantly confronts upper tier sheep, needs to be tested, even though it is difficult to imagine practical solutions to the phenomenon in the live sheep trade.
CHAPTER 8

DISCUSSION, SUMMARY AND CONCLUSIONS

The raison d'être for this research was to provide knowledge to help address the animal welfare debate surrounding the live sheep trade. The debaters demanded information about mortality rates and causes of death in the trade, and yet even the basic facts were unknown before this study commenced.

An epidemiologic approach was taken to the problem. Firstly, existing data sources were analysed (ship Masters’ Reports, Chapter 3) and basic observational studies were performed (VOY1 to VOY3, Chapter 4), which determined the magnitude of the problem and identified the major causes of deaths. More refined observational studies and specific designed experiments were then undertaken, in the feedlots and on board ships. These studies investigated the etiology of the inanition syndrome (Chapter 5), clarified the epidemiology of salmonellosis outbreaks (Chapter 6), and confirmed and tested hypotheses regarding the tier effect on shipboard mortality rates (Chapter 7).

8.1 Key Findings

8.1.1 Mortality Rates and Causes of Death of Sheep in the Live Sheep Trade

The research showed that most shipments of live sheep from Victoria suffered whole voyage mortality rates of between 1% and 4%, with an average of around 2½%. These rates were similar to those reported in parallel from Western Australia. There was no evidence that mortality rates were declining through time.
The major causes of mortalities were established. A syndrome associated with inanition was the leading cause of death, and gastro-enteric salmonellosis was the second most common. There were indications that the inanition syndrome also caused severe weight loss in up to 5% of the surviving sheep. Both the inanition syndrome and salmonellosis appeared to be endemic in the trade; they were the leading causes of death in every shipment studied. Together the two diseases accounted for approximately three quarters of all deaths, a picture which was repeated in Western Australian studies, but which differed from New Zealand findings where pneumonia is a major cause of death. Without inanition and salmonellosis, mortality rates in the Australian trade would approach normal background rates for adult sheep on farms.

The study provided evidence that a third major cause of death was a hyperthermia-related syndrome which occurred particularly in Middle Eastern waters during the northern hemisphere summer. These episodes involved the sudden onset of high mortalities in which sometimes thousands of sheep died rapidly on board ship.

8.1.2 The Inanition Syndrome

One of the major achievements of the study has been the broad elucidation of the inanition syndrome, which was the largest cause of death in the trade. The research has uncovered much evidence that the essential lesion in this condition is a lack of food intake right from the start of the export process. The pathology of the syndrome strongly suggested long term starvation; the rumens were empty and atrophied and all the attendant signs could be related to starvation. The prolonged epidemic curves of inanition mortalities which were described (4.3), were typical of a disease with a long latent period. Risk factor analyses showed that poor feeding behaviour in the assembly feedlot was associated with the syndrome, and serum biochemical changes confirmed that starvation began in some cases as early as the first few days after arrival in the

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feedlot. Many of these findings have been confirmed in the parallel Western Australian studies.

Biochemical profiles taken during the course of the inanition syndrome indicated that hepatic damage commonly occurred, and it was hypothesised that this injury may have arisen from excessive fat mobilisation which could damage hepatocytes (Bouchat et al 1981; Reid et al 1983; Walker et al 1984; Jubb et al 1985). It seemed likely that there were several causes of death possible with the syndrome, as indicated by diverse terminal lesions including uraemia and hypomagnesaemia.

Severe weight loss in surviving sheep was a related problem uncovered by the research. This was a new area of loss for the industry to consider. In particular, there was the possibility that inanition deaths were likely to continue after the sheep arrived in the Middle East. This is supported by the relatively high mortality rates that have been observed there (Beeby 1989; Scharp 1992). At the least, the sheep with severe weight loss would be less than fit for slaughter and would probably present a severe product quality problem in the trade. This area has not yet been investigated.

The small hospital pen trial provided a definite lead as to the real cause of the inanition syndrome. Sheep which appeared to be long term sufferers from inanition were seen to eat hay readily. The hypothesis was that the inanition syndrome resulted from failure to recognise the pelleted shipboard ration as food. While there is little precedent for recognition problems alone to cause starvation, it is accepted that unfamiliar foods are often refused by sheep (Arnold 1964, 1970; Arnold et al 1980; Chapple and Lynch 1986). The process of adapting to feed involves a distinct learning phase which can easily be compromised by psychosocial effects (Matthews and Kilgour 1979; Chapple and Wodzicka-Tomaszewska 1987a, 1987b; Provenza and Balph 1987; Chapple and Lynch 1986). In the live sheep trade it is easy to imagine compromises to effective learning, especially with the major changes which sheep undergo in the first few days of the process. The hypothesis would be consistent with the marker bar associations which were observed (Chapter 4); it would be consistent with starvation
occurring from the early stages in the feedlot (Chapter 5); it would be consistent with the pathology of the syndrome (Chapter 2); it would be consistent with the observation that trucking stress was associated with inanition (Chapter 4); and it would explain the eager feeding behaviour observed when affected sheep were offered a familiar food like hay (Chapter 5). The hypothesis relates to the elementary cause of the inanition syndrome, and as such I believe it should be a high priority for further research. Since the conclusion of this thesis, a follow-on study involving different physical forms of food has shown that the closer food is to its normal unprocessed form, the lower the incidence of inanition (Hodge et al 1991). This is a potential breakthrough in understanding the true etiology of the inanition syndrome.

8.1.3 Salmonellosis

In comparison to the inanition syndrome, salmonellosis in the live sheep trade was a far more straightforward disease. My research has shown that salmonellosis occurred as rise and fall epidemics, beginning in the feedlot and finishing about half way through the voyage. This is supported by other recent descriptions of salmonellosis in the trade (Higgs et al 1993). Results from the exceptional VOY4, which had a prolonged feedlot period, even indicated that the epidemics may pass in a set time frame whether the sheep are on board ship or not (Chapter 6). In this regard, salmonellosis appeared to be a self-limiting disease in the live sheep trade.

The VOY4 study (Chapter 6) showed the classic pattern of an explosive increase in excretion straight after transportation, as seen in numerous other intensive sheep husbandry situations (Moule and Young 1951; Grau et al 1968; Hails 1968; Williams and Newell 1970). When combined with the relative crowding, the on-ground feeding of loose hay, and the presence of open ground water in many feedlot paddocks, it is not surprising that salmonellosis epidemics result. Further logical connections were uncovered; salmonellosis was associated with trucking stress, poor feeding behaviour,
and a lack of prior experience with hay (Chapter 4). Another epidemiologic possibility suggested by several findings in Chapter 6, was that salmonella infection may persist in the feedlot environment between consignments of sheep, which could infect incoming batches and generate epidemics. No direct proof of this hypothesis was obtained, but the circumstantial evidence was strong.

Control strategies for salmonellosis should now be developed. Regardless of the source of infection, an improvement in feedlot hygiene should be efficacious (Blood and Radostits 1989). Hay could be fed from hay racks, instead of on the ground, so as to keep it high and dry. Ground water could be removed by careful drainage and the reticulated water which is used could be supplied in foul-proof troughs.

8.1.4 Heat Crashes

Indirect evidence was obtained which supported existing anecdotes that heat causes sporadic, high mortality episodes on board ships. The episodes were evident in data from ship Masters’ Reports. The time and place at which they occurred (hot season in the Arabian Gulf), and the comments by ship Masters’ describing severe heat and/or humidity were evidence that hyperthermia was the cause. The weather conditions that have been recorded in sheep houses (Hamilton et al 1961; Gardiner and Craig 1970; Truscott 1977) were certainly similar to those described as dangerous in the literature (Lee and Robinson 1941; Robinson and Lee 1947; Syme 1985), and the clinical signs of severe heat stress were observed on board ships (Chapter 4 and footnote in Chapter 3). The episodes affected 6 out of 21 shipments (29%) which entered the Arabian Gulf in the hot season during the five years surveyed, and they accounted for 9% of all shipboard deaths in that period.

The heat crash phenomenon requires further research if its cause is to be elucidated, but the work would be difficult to perform given the sporadic nature of the
events. Even without fully understanding the aetiology of heat crashes, it may be possible to devise interim preventive measures. Weather warnings in the danger areas may allow ships to dodge the problem if alternate ports or routes could be arranged at short notice. Alternatively, the cessation of shipments in the danger season (May to September) could be an option.

8.1.5 Poor Feeding Behaviour as a Risk Factor

One of the strongest and most consistent risk factors associated with both the major causes of mortality was poor feeding behaviour in the assembly feedlot. This finding is strongly supported by recent publications from the parallel study in Western Australia (Norris et al. 1989a, 1989b). Both predictors and effects of poor feeding behaviour were used with similar results, namely the marker bar and serum biochemical tests. Attempts to improve the adaptation of sheep to the pelleted shipboard rations could improve both mortality rate and weight changes during export. Many recent attempts, including better trough access (Hodge et al. 1991), more food (McDonald et al. 1990), and longer feedlotting (Norris et al. 1992) have so far failed. Clearly one area to address is the learning process which sheep must go through during adaptation. Different physical forms of ration, or different feeding regimes to facilitate learning, or the development of systems to detect and remove non-adapted sheep before loading, could all be possible solutions.

8.1.6 The Stress of Transition from Farm to Feedlot as a Risk Factor

In the group risk factor analysis of VOY1 to VOY3 (Chapter 4), factors associated with the stress of the trucking journey from farm to feedlot were associated with subsequent mortalities from inanition and salmonellosis. Factors which would lead
to increased stress during trucking were associated with higher mortality rates. At first glance this may seem like a fanciful connection, considering that trucking is such a common occurrence in modern agriculture, and that it occurs in such a brief span of time at the very beginning of the export process. However, the theory needs expounding.

There are many precedents in trucking research to indicate that poor trucking experiences may severely affect animals. Clinical, biochemical and behavioural changes may all occur for periods up to several days after trucking (Shaw and Nichols 1964; Hails 1968; Crookshank et al 1979; Kelley et al 1981; Galyean et al 1981). One specific effect, which is supported by anecdote in the live sheep trade, is that animals which have been stressed by trucking will lie down for prolonged periods over several days after the journey (Bisschop 1961; Sutton et al 1967; Tollersrud et al 1971). If trucking causes such adverse physiological effects in some sheep on arrival at the feedlot, it is conceivable that the whole adaptation process for these animals is jeopardised, especially considering the relatively short feedlot period that is given for adaptation.

The hypothesis fits well with a number of observations. For example, if sheep were compromised in their learning ability during the feedlot period, they may not learn about pellets before they get on board ship. This could be the foundation of the inanition syndrome. Sheep which were stressed on arrival at the feedlot would also be more susceptible to salmonellosis. Also, starvation in the feedlot would exacerbate salmonellosis. The hypothesis also provides a possible explanation for the *Company T* effect described in the Masters’ Report analyses in Chapter 3. One of the differences about this Company, which had significantly lower mortality rates than others, was its rigorous attitude to culling sheep *on arrival* at the feedlot. If that practice effectively removed those animals which had had stressful trucking experiences, which was one of its major aims, then it may have helped the Company achieve such low mortality rates. The theory appears worthy of further exploration.
8.1.7 The Tier Effect

My research discovered the "tier effect", in which higher mortality rates occurred in upper tier pens than lower tier pens on board ship. The magnitude of the increase in mortality was around two-fold in the five data sets in which it was described. Given that the industry-wide mortality rate is around 2.6%, and half of all sheep are housed on upper tiers, the mortality rate attributable to the tier effect alone would be approximately 0.9%, or one third of all deaths.

The investigation of tier effects on cause-specific mortality rates did not show clearly whether the effect influences all causes equally. However, it did appear that inanition deaths were increased in upper tiers. There was also evidence that feeding behaviour in general was compromised in upper tiers compared to lower tiers. Specifically, upper tier sheep were slower to adapt to feeding during the voyage, slower at eating, and present at the feed trough less often, than sheep in lower tiers. These findings implied that the tier effect exerted a general influence on the population which especially affects feeding behaviour, and this was probably the force which mediated the mortality effect. There may also be an, as yet undetected, effect on water consumption, since this was not directly measured in any of my studies. However, no indications of chronic dehydration were noted in the necropsy findings, or in the biochemical case studies reported in Chapter 5. The findings also suggested that there may be weight change effects between tiers, as yet undiscovered. If such effects exist, they could easily be more important economically than the mortality effects.

The cause of the tier effect remains unknown. One hypothesised cause - higher light intensity in upper tiers - was formally tested and rejected by a field trial (Chapter 7). A remaining possibility concerns the "visual cliff" which confronts sheep as they look through the sides of their pen, such as when eating. Sheep have notoriously poor depth perception, and are known for their habit of fearing even a shadow as they leap
through gateways (Grandin 1980). The theory is therefore plausible, but it is yet to be tested.

8.2 Wider Implications of the Research

8.2.1 For the Social Debate On the Live Sheep Trade

My research has provided quantitative information on the animal health and welfare problems in the live sheep trade. This will facilitate the social debate. There is no doubt that more sheep die in the live sheep trade than would otherwise die in a local slaughter situation. This must be balanced against the loss of trade that would occur if live sheep export was prevented. At the time of writing, market intelligence indicates that frozen meat trading is unlikely to ever replace the live sheep trade because of the Middle East customer’s requirement for meat to be freshly killed. However, moves to improve the methods of meat preservation and transport could provide a solution. If meat could be transported to the Middle East by sea and still arrive in an acceptably "fresh" condition, the Middle East customer would get cheaper product because of the lower transport costs and lower losses, and the sheep welfare issue would be resolved. A key factor in this scenario is the extent to which the products of any new meat preservation technology were accepted by the Middle East.

The new knowledge that the research has generated on causes of death will also allow better value judgements on the current animal welfare situation. In this regard, the inanition syndrome is a peculiar case. My evidence that sheep with inanition may simply be starving for recognisable food, could make this syndrome a more potent welfare concern than more ordinary diseases because starvation is such an emotive issue. Another aspect of the inanition syndrome which will be important in welfare terms is
the additional impact of severe weight losses and/or further deaths in the Middle East which are likely to be involved.

The confirmation that heat crashes occur will also have a significant impact on the animal welfare debate. Although this condition occurred sporadically, and ranked only third in the total causes of death, the circumstances of death from hyperthermia are again a strongly emotive picture and one which could not be easily justified on any economic grounds.

For all these reasons, it will be important, in order for the research to have an impact on the community debate, to deliver the results effectively to the key debaters. This will be a specific task in completing the work.*

8.2.2 For the Live Sheep Export Industry

The live sheep export industry will be able to use much of the new knowledge generated by this research. Salmonellosis control would be one of the more compelling areas for initiative. Even without definitive answers on the role of environmental reservoirs of salmonella infection, there appears to be scope for reducing the incidence of the disease by breaking the oral-faecal cycle. Both the feeding of hay in hay racks instead of loose on the ground, and the careful drainage of ground water should be effective if implemented. This recommendation should be acted on by feedlot owners.

A practical exploration of the practices used by Company T which may cause its mortality rates to be lower than all other Companies would also be in order. The main

*Both during and after the research a comprehensive communication and extension program has been undertaken, targeting the key decision makers with publications and presentations as listed at the beginning of the thesis. The program included a professionally produced booklet containing the essential results, copies of which can be obtained by writing to me.
hypothesis resulting from the research relates to the Company's quality control practices applied to sheep on arrival at the feedlot. In particular, it may pay other Companies to experiment with more rigorous culling of sheep which appear to have been adversely affected by transport to the feedlot. The managers of the export companies should act on this recommendation.

Finally, the live sheep export industry should examine ways in which heat crashes can be avoided. Weather prediction technology may be able to be applied to forewarn of dangerously hot and humid weather where sheep ships may pass. Ships may be able to be directed to alternate ports or travel via alternate routes in these circumstances. A more extreme solution may be to avoid the dangerous routes, or avoid shipping altogether, during the hot season. This recommendation will require joint action by the regulators of international shipping and the live sheep export companies collectively.

8.2.3 For Further Research in the Live Sheep Trade

A number of areas require further research, but some are more pressing than others. The development of improved methods for meat preservation and transport could provide a lateral way around the whole problem of animal welfare in the live sheep trade. Given the effectiveness of the solution it could offer, and the long term nature of the research, this area should be given a high priority and a reasonable time frame in which to achieve results. It would be important that market research, and possibly also active promotion of the technology, occurs very early in this program to facilitate its acceptance by Middle Eastern consumers.

More basic research is required into health problems in sheep after their arrival in the Middle East. This area is important not only to complete the whole animal health picture of the live sheep trade, but also to address and understand the problems of the Middle East principals, since they are the real decision makers in the live sheep trade.
If the inanition syndrome dominates even more in the post-arrival phase than in the shipping phase, as the epidemiologic results suggest, then that would increase the importance of the syndrome in general. It would also encourage the development of preventive measures which could be applied very early during the export process with potentially great and lasting effect.*

The third priority area for research is the investigation of environmental reservoirs of salmonella infection in the feedlots. If such reservoirs exist, their potential contribution to the total of salmonellosis cases may be large (as indicated in Chapter 6), and as such their control should be attempted.

Further research on the role of stress during the introduction of sheep into the live sheep trade also seems warranted. Physiologic stress may be an important determinant of mortalities generally in the live sheep trade. While only hypothetical, the concept is attractive, coherent and consistent with many of the observations made about mortalities and their associations.

Finally, a test should be made of the hypothesis that the tier effect may be caused by fear of the visual cliff which confronts sheep in upper tier pens. While it is difficult to imagine a practical solution to this phenomenon, the magnitude of its effect on mortalities makes it worthy of exploration.

*Since this research was completed, a project to describe health problems in the Middle East has been commissioned.
8.3 Conclusion

Knowledge has been advanced by this research. Its major contribution has been to describe the animal health situation in the complex animal husbandry system of the live sheep trade. The inanition syndrome has been described and its pathogenesis elucidated. The epidemiology of salmonellosis in the live sheep trade has been explored. The existence of heat crashes has been confirmed. For some of these problems potential solutions have been developed, some to the point of implementation while others require more research. All these advances have an impact on the animal welfare debate involving the live sheep trade, putting it on a firmer base, and clearly moving it towards a resolution.
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