Project Report: Quantitative risk assessment of *Salmonella* in sheep meat produced in New Zealand.

Introduction

The research project "A quantitative risk assessment of *Salmonella* in sheep meat produced in New Zealand" was initiated with a meeting in Gore in July 2000, attended by a wide range of stakeholder groups. The meeting followed agreement that *Salmonella* Brandenburg was an important issue and the securing of majority sponsorship by Meat New Zealand. The research project has a three-year duration. This report presents the information gathered over one and a half years and assesses the progress towards achieving both the short and long-term goals agreed on at the Gore meeting.

The first section of this report outlines the major research activities in each of the project areas to date and summarises results where these have been completed. The second section examines the results against the overall and short-term goals of the project, and outlines the areas where data is being sought to complete the project.

Part I: Project Research

The Gore meeting report set out the structure for administration of the project. Although this has changed slightly to better meet the needs of the project, the modular approach has been maintained. The project consists of the following modules:

Farm module
Processing module
Storage/distribution/retail module
Consumer module

The research and results are presented under each of these headings below. Research leaders are producing individual study reports containing more detail and references. Each of the studies provides not only data that can be incorporated into the quantitative risk assessment model, but also information on *Salmonella* (and *S.* Brandenburg in particular) from animals on the farm to the meat purchased by the consumer. The focus of most of the discussions in this document will be on this latter aspect.

1.1 Farm module

a) ***Salmonella* prevalence in lambs and ewes.**

The purpose of this study was to examine the relationship between the occurrence of clinical epidemics of *S.* Brandenburg abortion in sheep flocks and the risk of *Salmonella* infection in ewes and lambs going to slaughter. This was done by comparing the prevalence of *Salmonella* in groups of ewes and lambs on farms that experienced clinical outbreaks of *S.* Brandenburg abortion with that on farms in the same region that had no disease outbreak during the 2000

season. Prevalence was examined in faecal samples collected from groups of ewes or lambs on farms, and in the caecal samples collected from the same groups of animals following slaughter. The sampling protocol was repeated on the same flocks in December/January and February/March (early and late season) as it was considered likely that risk would be related to time since the disease outbreaks. Rectal faecal samples were collected from 50 lambs and 50 ewes drafted for slaughter, for each of 4 case and 4 control farms. Due to logistic problems, lower numbers of ewes were sampled. Case farms were purposively selected based on the occurrence of severe epidemics, on the presumption that this would reflect the 'worst case scenario' with respect to risk of contamination, and would be more likely to yield positive results.

Results

The results of on-farm (faecal) and post-slaughter (caecal) sampling are presented in Table 1 and Table 2 respectively. All isolates were confirmed as *Salmonella* Brandenburg using a methodology standardised across the project, and a proportion were further typed to confirm the isolates were the outbreak strain responsible for the cases in Otago/Southland.

Table 1: Summary of the proportion of animals positive for *Salmonella* spp. by faecal culture of on-farm samples during phases I (Dec/Jan) and II (Feb/Mar)

	Phase I		Phase II	
	Lambs (n/N)	**Ewes**	**Lambs**	**Ewes**
Affected Farms	24/200 (12%)	28/150 (18.7%)	5/200 (2.5%)	4/150 (2.7%)
Control Farms	8/200 (4.0%)	8/200 (4.0%)	0/200 (0.0%)	1/129 (0.8%)
Totals	32/400	36/350	5/400	5/279

Table 2: Proportions of animals positive for *Salmonella* spp. by culture of caecal contents collected at the abattoir during phases I (Dec/Jan) and II (Feb/Mar)

	Phase I		Phase II	
	Lambs	**Ewes**	**Lambs**	**Ewes**
Affected Farms	18/200 (9.0%)	31/150 (20.7%)	0/200 (0.0%)	4/150 (2.7%)
Control Farms	0/200 (0.0%)	1/200 (0.5%)	1/198 (0.5%)	0/121 (0.0%)
Totals	18/400	33/350	1/398	4/271

Discussion

The most significant observations of the study were

- *Salmonella* Brandenburg was isolated from all case farms and 3 out of 4 control farms, and was isolated from all *Salmonella* positive samples (only one other serovar detected).
- The proportion of positive samples declined markedly between the first and second samplings, indicating that risk of contamination is highest early in the season
- *Salmonella* were more frequently detected in case farms than non-case farms, although positive results were strongly clustered within farms.
- There was minimal difference in isolation rates between lambs and cull ewes, indicating that elevated risk occurs in both classes of stock.
- Results of sampling on-farm and after slaughter were similar, suggesting that transport and lairage did not markedly affect the detection of the organism. Effects on fleece contamination were not evaluated.

This pilot study was designed to obtain an indication of the potential implications of disease epidemics with respect to risk of foodborne contamination. Statistical analysis was not applied due to the purposive selection of farms and small number of farms studies. However, the results indicate that outbreaks on farm do have implications for risk of *Salmonella* detection in both ewes and lambs, and that the major period for concern is early in the season.

b) Retrospective case-control study of Otago/Southland farms

This study set out to compare environmental, management and animal factors in farms clinically affected with *S.* Brandenburg, and unaffected flocks using a mail-out questionnaire. Farmers included in the study were clients of three veterinary practices in the affected regions of the South Island (Otago and Southland). Veterinary practices identified case farms where they had diagnosed *S.* Brandenburg disease during the 2000 season. They also provided contact details of all sheep clients of their respective practices. Control farms (3 per case farm in each practice) were randomly selected from the client lists and questionnaires were mailed to all case (148) and randomly selected control farms (444). Questionnaires asked for information about animal and management factors at the flock and farm level for the 2000 lambing season. These potential risk factors were screened for association with the occurrence of disease outbreaks using univariate methods. Final models are presented as multivariate analyses, via logistic regression, using SPSS® and SAS®. Disease in hoggets was ignored in the analyses. Separate models were used for disease in mixed age and two-tooth mobs.

Results

Usable responses were received from 405 (68%) of farms, with the response rate being greater for case (81%) compared with control (64%) farms (Table 3).

Table 3: Summary of questionnaire response rate

Category	Questionnaires mailed	All Responses (Rate %)	Usable Responses (rate %)
Case	148	123 (83)	120 (81)
Control	444	322 (73)	285 (64)
Total	592	445 (75)	405 (68)

Among the 285 usable responses from selected control farms, 56 respondents reported that their flocks had been affected by *S.* Brandenburg disease during the 2000 season. These farms were reclassified as case farms for analysis, resulting in usable responses from 176 case and 229 control farms (Table 4).

Table 4: Summary of sheep numbers on respondent farms

Category	Farms (n)	Hogget (n)	TT and MA (n)
Case	176	108,324	449,232
Control	229	126,990	504,186
Total	405	235,314	953,418

Of the 176 affected farms, 172 (98%) reported disease affecting mixed age ewes, while only 8 farms (4.5%) reported *S.* Brandenburg disease in hoggets (Table 5). There was no farm on which disease occurred only in hoggets (Table 5).

Table 5: Pooled reported risk of abortions and death on affected farms

Mob	Farms	Ewes in affected mobs (n)	Diseased (%)	Mortality (%)
Hogget	8	3,115	5.2	1.0
TT	78	55,890	5.6	2.5
MA	172	272,425	4.3	2.4

Factors associated with the occurrence of outbreaks on farms

The most striking findings on univariate analysis were positive associations of occurrence of disease with use of strip grazing (odds ratio 6.9) and the occurrence of disease in 1999 (OR 3.9 for mixed age ewes; 2.9 for two tooths). The occurrence of disease in 1999 was excluded from the multivariate analyses as it is expected to be confounded with other explanatory risk factors and would be likely to obscure their impact on disease. Other variables with relatively strong associations were stocking density (increase risk at higher density), and terrain (higher risk on flat than hill land), and various feeding practices. The final model (Table 6) indicated that strip grazing (particularly with back fencing) has the strongest association with disease occurrence.

Table 6: Summary of logistic regression analysis of factors associated with occurrence of *S.* Brandenburg disease in mixed-age (MA) and two-tooth (TT) mobs (OR of 1 indicates no association).

MA Risk Factor	P Value	OR
Main effects		
[#] Ewe numbers	<0.001	1.037
Strip grazing (Yes + BF* vs No)	<0.001	9.8
(Yes - BF* vs No)	0.002	6.1
Terrain (Hill vs Flat)	0.001	0.35
Crop (Fed vs Not fed)	0.008	0.4
Interaction		
Crop*Terrain	0.081	2.6

TT Risk Factor	P Value	OR
Main effects		
Hay (Fed vs Not fed)	0.017	2
Strip grazing (Yes + BF* vs No)	0.019	4.3
(Yes + no BF* vs No)	0.157	3

*BF: Backfencing

#OR expressed per incremental increase
per 100 ewes

Factors associated with the severity of disease on farms

Binomial logistic regression analysis was conducted on affected farms only to identify factors that may be associated with increased severity of disease on affected farms. The final models for mixed age and tooth groups are given below (Tables 7a, 7b). Vaccination, which was not found to be associated with the odds of disease on farms in the previous analysis, was associated with reduced severity of disease.

Table 7a: Summary of binomial logistic regression analysis of factors associated with reported severity of *S.* Brandenburg disease in mixed-age ewes on affected farms.

MA Risk Factor	P Value	OR
Main effects		
Vaccination (Yes vs No)	0.002	0.36
Shear (<July vs>July)	0.025	0.63
Ram removed (<July vs >July)	0.08	1.4
Interactions		
Shear*Vaccinate	0.09	0.46

Table 7b: Summary of binomial logistic regression analysis of factors associated with reported severity of *S.* Brandenburg disease in two-tooths on affected farms.

TT Risk Factor	P Value	OR
Main effects		
Vaccination (Yes vs No)	0.003	0.46
Hay (Fed vs Not fed)	0.02	4.4
Crutch (>July vs <July)	0.04	0.6
Strip grazing (Yes + no BF vs No)	0.06	5.1
(Yes - BF vs No)	0.09	3.9

Discussion

This was an exploratory study to attempt to identify factors that may be associated with increased risk and severity of *S.* Brandenburg disease. The nature of the study (mail questionnaire) and methods for classifying farms (veterinary/farmer opinion) need to be kept in mind when interpreting the results. The findings of the study should be viewed as indicators of possible factors worthy of further investigation rather than proven risk factors for the disease. However, the study has provided several findings that help advance our understanding of the problem.

The observation that the occurrence of an epidemic *S.* Brandenburg disease in 1999 substantially increased the odds of an outbreak occurring in 2000 is important in two perspectives. Firstly, it indicates that there is no rapid substantial increase in flock immunity following an outbreak, and that farms that have experienced disease may continue to do so. However, the role of flock immunity in the longer term (i.e. following repeated annual outbreaks) remains to be determined. Secondly, the recurrent pattern of disease on certain farms suggests the importance of farm-level factors affecting risk of disease occurrence. Exposure to *S.* Brandenburg is obviously a necessary event for disease to occur, and the exposure status of the unaffected farms is largely unknown. However, several control farms have previously experienced disease (i.e. high probability of exposure), and there is mounting evidence that *S.* Brandenburg is now widely distributed in the ecosystem of this region. In the companion study reported above (Prevalence of *Salmonella* in lambs and ewes), *S.* Brandenburg was identified in sheep from all 4 control farms selected because of a firm history of absence from clinical disease. Although magnitude of exposure must have some impact on the risk of outbreaks, we suggest that most farms in the epidemic area are likely to harbour the organism and that risk of outbreaks will be largely attributable to factors other than the presence or absence of the organism on farms. That is, environmental and management factors may play an important role in explaining the elevated risk of recurrent outbreaks in farms that have previously experienced the disease.

The most striking finding (based on strength of association) was the association of strip grazing on odds of disease, and this practice is the most likely factor to be causally associated with outbreaks. In addition, the apparently increased risk associated with back fencing when strip grazing further supports a causal role. This is based on the strong associations observed and the knowledge that high animal density favours transmission of *Salmonella* in other species. However, the data also indicate that strip grazing is a preferred management approach on sheep farms in the region (practised on approximately 85%% of respondent farms) and many farms in the study that reported that they strip grazed had not yet experienced an outbreak of disease.

Clearly, discouragement of strip grazing (or back fencing) is an option for mitigating the risk of disease in the industry. However, because the practice is so common, better understanding of factors that may explain patterns of disease on strip grazing farms is warranted. This could lead to management approaches compatible with strip grazing that reduce risk of disease. The role of various feed related factors (e.g. crop, hay, etc) is unknown and it must be remembered that such practices are likely to be confounded with stocking density, pasture quality and availability etc. We are now commencing more detailed studies to obtain a better understanding of management practices on strip grazing farms in relation to risk of disease. Further spatial and multivariate analyses that we are conducting may yield further insight into the relative importance of these and other factors in explaining the patterns of outbreaks observed.

1.2   Processing module

a)   Prevalence and concentration of *Salmonella* on pelted carcasses.

Six of the eight mobs sampled in the on-farm prevalence study were followed through into the processing plant. Carcasses from three case and three control farms were sampled in December 2000 and in February 2001. 51 ewes and 51 lambs from each farm were split into 3 groups of 17, each group being sampled at a different point in the processing chain: the slaughter floor, the marshalling floor (prior to cooling), and the boning room. The purpose of sampling at different points was to examine the effect of processing on the prevalence and concentration of *Salmonella*. Fleece samples from the 3 control farms were also taken in February, on farm and prior to slaughter, to look at possible cross-contamination during transport and lairage.

Sampling was also carried out in February 2001 in two geographically separate North Island areas (outside of the *S*. Brandenburg endemic area), to obtain a New Zealand wide picture of the level of all *Salmonella* species on carcasses. 102 ewe carcasses and 101 lamb carcasses were sampled after pelting on the slaughter floor only, to maximise the possibility of finding *Salmonella*.

Results

The results for the case and control farms in the *S*. Brandenburg area are summarised in Tables 8 and 9. These results were all confirmed to the level of *S*. Brandenburg using the agreed methodology, with a proportion analysed further to confirm isolates were the same as the outbreak strain. The numbers of bacteria were also estimated by the 'most probable number' (MPN) technique, the preferred option for samples with low numbers of bacteria.

	Case positives		Non-case positives	
	Lambs	Ewes	Lambs	Ewes
Slaughter floor	34/51 (66.7%)	17/51 (33.3%)	32/51 (62.7%)	0/51 (0.0%)
Cooling floor	21/51 (41.2%)	10/51 (19.6%)	14/51 (27.5%)	0/51 (0.0%)
Boning room	0/51 (0.0%)	0/51 (0.0%)	0/51 (0.0%)	0/51 (0.0%)

Table 8: Processing results – December 2000

	Case positives		Non-case positives	
	Lambs	Ewes	Lambs	Ewes
Fleece (farm)	x	x	0/30 (0.0%)	0/30 (0.0%)
Fleece (works)	x	x	0/30 (0.0%)	0/20 (0.0%)
Slaughter floor	1/51 (2.0%)	0/51 (0.0%)	2/51 (3.9%)	2/51 (3.9%)
Cooling floor	3/51 (5.9%)	0/51 (0.0%)	0/51 (0.0%)	0/51 (0.0%)
Boning room	0/51 (0.0%)	0/51 (0.0%)	0/51 (0.0%)	0/51 (0.0%)

Table 9: Processing results – February 2001   x = not sampled

In all the groups in which positive isolates were made, the numbers assessed to be present were wide in range, usually from less than one organism per cm^2 to more than 70 or 300 organisms per cm^2 (the upper limit of the MPN techniques used in December and February respectively).

No *Salmonella* of any species were isolated from the samples taken from the 102 ewe and 101 lamb carcasses in the North Island.

Discussion

One of the main features of the December results was the high prevalence of *S.* Brandenburg on lamb carcasses at slaughter, both in the case and non-case farms. The carcasses of lambs from non-case farms returned almost as many positive samples as those from case farms, despite having a consistently lower faecal and caecal prevalence. This may be due to cross-contamination at the transport, lairage or processing level. Ewes from case farms overall returned half as many positive samples as lambs from case farms, yet *Salmonella* was not detected on the ewe carcasses from non-case farms. In all groups, positives decreased substantially over the process from slaughter floor to cooling floor to boning room.

Results from the February round of sampling show a significant decrease in the number of isolates, as seen in the on-farm module. Given Feb/March represents the peak time for lamb processing, this is a particularly welcome result. One possible reason for the decrease could be the passage of time since the peak of the clinical cases (August). The overall lack of isolates in February precludes much analysis between flocks and/or stock classes. This is also true of the negative fleece results from control farm animals comparing fleece contamination pre and post transport/lairage, which were sought to provide information on cross-contamination.

The lack of *Salmonella* isolates from carcasses in the North Island reflects the fact that it is outside the area where *S.* Brandenburg occurs and that the prevalence of other *Salmonella* serotypes is not high. The ability to move stock between islands for slaughter however means that *S.* Brandenburg could potentially be isolated from carcasses processed in the North Island.

No positives were found in the boning room samples in either sampling period, although it is important to note the sampling area in December ($100cm^2$ on legs only) was less than and did not reflect the exact same sites as the other two sampling areas. The sample sites were selected on the assumption that redistribution would occur during boning. On the basis of the December results the range and size of boning room cut samples were increased for the February trial (legs, flanks and shoulders with an area of 1258cm2 in lambs and 1720cm2 in ewes). However again no positives were found, perhaps due this time to the overall drop-off in prevalence. Subsequent studies in the processing module (see b). below) and at the retail level (1.3a) sought to clarify the levels that could be occurring in end product in the late spring/early summer.

b)   Prevalence and concentration of *Salmonella* on freshly pelted case-farm carcasses and boned cuts

This study aimed to clarify the perceived decrease in *Salmonella* prevalence from slaughter floor to boning room observed in the previous study. To maximise the possibility of isolates from the boning room samples, the study was done in November/December 2001, using only lambs from case farms

(farms that had experienced abortions in the current season). Also a larger sampling area was used in the boning room compared to either of the previous studies (see a). above).

Ten lambs from each of ten case farms were included in the trial. Sampling was done at two points (2 groups of 5 lambs) – during pelt removal and on specific cuts during boning. The sampling on the marshalling floor was not done in this trial as there was sufficient data from the earlier trials.

Results

Results from this study are presented in Table 10. All of these isolates were confirmed as *S*. Brandenburg by serotyping and shown by PFGE to be the same type causing abortions.

	Total samples	Case positives
		Lambs
Slaughter floor	53	21(39.6%)
Boning room	53	2 (3.8%)

Table 10: Processing results: Case farms– December 2001

Discussion

The prevalence of *Salmonella* on carcasses at slaughter was still high, but lower than the previous season (39.6% vs 66.7%) for lambs from case farms. This could be due to a lower general infection rate reported for *S*. Brandenburg in the 2001 season. Numbers of bacteria were generally very low, from the lower limit of detection (0.2 cfu/100cm^2) to a high of >3070 cfu/100cm^2 in one sample.

S. Brandenburg was isolated from 3.8% of cuts in the boning room, in contrast to previous negative results. This can be attributed to using an increased sampling area in the boning room and occurred despite the lower number of positives on the carcasses at slaughter, and the very low numbers of *Salmonella* per unit area.

The drop in the isolation rate between the slaughter floor and boning room (90.4%) might be attributed to the dilution/redistribution effect of the boning process and/or the drying effect of the chillers in the processing plant. Drying of carcass surfaces during chilling could have a two-fold effect on bacteria:
- Reduce viability.
- Increase adhesion, which would make it difficult to remove during swab sampling.

Some of these aspects will be examined in greater detail in the 2002 phase of the processing module.

c)   **Prevalence and concentration of *Salmonella* on randomly sampled pelted carcasses**

The case/control format employed for the above studies provided information on the differences between the extreme groups and the effects of cross-contamination events on carcass levels of *Salmonella*. However, the animals sampled weren't representative of the general population and therefore estimates of the total carcass load of *Salmonella* entering the food chain couldn't be made.   To achieve this aim carcasses were randomly selected for sampling within the processing plant.
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The study was done in November/December 2001. 120 ovine carcasses were selected over a period of 2 weeks. Sampling was done on the slaughter floor only, immediately after pelt removal. The sampling sites and microbiological methodology were the same as for the earlier studies.

This was done both in the *S*. Brandenburg endemic area and in two North Island plants, where *S*. Brandenburg hasn't been reported.

Results

Results from this study are presented in Table 11 for the South Island and Table 12 for the North Island.

	Lambs		Ewes	
	Total samples	Positives	Total samples	Positives
Slaughter floor	73	11(15.1%)	47	10 (21.3%)

Table 11 - Processing results: Randomly selected carcasses South Island

Most positive isolates were confirmed as *S*. Brandenburg, but there were also some other salmonellae including *S*. Typhimurium.

	Lambs		Ewes	
	Total samples	Positives	Total samples	Positives
Slaughter floor Plant 1	35	1(2.9%)	25	2(8.0%)
Slaughter floor Plant 2	29	0	31	0

Table 12 - Processing results: Randomly selected carcasses North Island

The three positive isolates from the North Island were all confirmed as *S*.Hindmarsh

The bacterial numbers in both South and North Island random carcasses were quite low (from 0.2/100Cm2 to 11.2 cfu/100cm2 in the South and 0.2/100Cm2 to 41.9/100cm2 in the North).

Discussion

The carcass contamination rates of randomly selected animals from the *S*.Brandenburg endemic area were still moderately high although, as expected, substantially lower than case farms. There is also a slightly higher incidence of positives among ewes than lambs (21.3% vs 15.1%). These figures will provide baseline data on carcass prevalence for the model, and will be related to the incidence of clinical cases by way of a questionnaire survey sent to farmers who provided stock for slaughter around the time of the study. In subsequent seasons the relationship between on farm incidence and carcass contamination will be investigated further.

The isolation of *S*. Typhimurium for the first time this year may be indicative of the higher incidence of this pathogen, especially among birds such as sparrows. It is also the predominant type found in human cases this year.

This year saw positive isolates from the North Island study. These were of the animal enteric type, *S*. Hindmarsh, which is not unexpected.

1.3   Storage/distribution/retail module

a)   Retail sampling of sheep meat

The aim of this study was to provide data on prevalence and concentration of *Salmonella* at another point in the chain from the farm to consumer, to validate the model results at this point. The results were expected to reflect any growth or revival of bacteria from the almost zero numbers found in the boning room studies. Sufficient data collected at this point could also be used to directly model the risk to the consumer, given further information on handling, consumption and susceptibility of the population.

Six hundred sheep meat samples (300 lamb fore-leg shanks and 300 lamb leg chops) were purchased from retail butchers, supermarkets and retail outlets at meat works in the Otago and Southland area over a three week period, November/December 2001. Lamb was chosen as the prevalence was higher on lamb in the earlier carcass studies. The samples were kept chilled prior to laboratory analysis for *Salmonella.*

Results

Out of six hundred samples tested, 17 samples (2.8%) were positive for *Salmonella*. All the isolates were confirmed by PFGE to be the *Salmonella* Brandenburg strain causing the disease in the area. Fifteen of the 17 samples were lamb leg shanks, 2 were leg chops.

The concentration of *Salmonella* on the meat cuts, enumerated by the MPN method, was very low. Out of 15 shanks, 12 samples had a count of <6 MPN per sample. Two shanks enumerated 8 MPN/sample and one had a count of 18 MPN per sample. Of the two positive chops, one had a count of <6 MPN/sample and the other 18 MPN/sample.

Although 17 samples tested positive for *S*. Brandenburg in this survey, the samples seem to have been clustered in 9 groups. The samples in each group were purchased from the same retail shop on the same day and time. One obvious relationship here is that the samples from within each group could have come from the same batch of contaminated carcasses or been contaminated during cut processing from within the retail outlets.

Discussion

This project showed that *S*. Brandenburg is present in retail meat cuts but the numbers from positive samples were low. However the prevalence was greater than that seen in boning room samples from within the processing plant. The reason for this is unknown and the small number of samples and the clustering of the results add uncertainty to any supposition. If the earlier hypothesis that the drying conditions in the plant make the recovery of still viable bacteria difficult is substantiated, then the recovery of a greater number of isolates may be due to the more hydrating retail conditions, or the adaptability of the bacteria.

The combination of the low prevalence and concentration of *S*. Brandenburg in retail lamb cuts suggests that the risk to the NZ consumer from sheep meat is low, especially when the meat will be cooked prior to consumption. However to answer this question more precisely the data gained in this study will be modelled in the quantitative risk assessment, which will incorporate consumer handling and the dose/response relationship. Availability of epidemiological information from the human case control study (see consumer module) may also help in assessing this risk.

1.4 Consumer module

a)   Human case-control study

This study started collecting data in late January 2002. The data collection phase will run for a year, and results should be available mid-2003. The aim of the study is to quantify the incidence of human infection with *Salmonella* species, in particular *S.* Brandenburg, and to estimate the contribution of New Zealand sheep meat consumption to this incidence. The results will also be compared to the outputs of the risk assessment model, which should be similar if the model is valid.

The study is using a standard case-control methodology. *Cases* (individuals diagnosed with salmonellosis) are interviewed to elicit information about a range of risk exposures for salmonellosis. Cases are matched with *controls* (individuals from general population without salmonellosis), who are also interviewed about salmonellosis risk exposures.

Cases of *S* Brandenburg infection are eligible for inclusion as well as a random selection of cases due to other *Salmonella* types, up to a study total of 282 cases. Controls are matched with cases by age group and geographic region of residence.

Cases and controls are interviewed by phone using a standardised questionnaire. The questionnaire contains questions about demographic information and exposure to salmonellosis risk factors, including sheep meat consumption and occupational exposure to meat and farm animals. The responses will be compared, and the comparison will provide a quantitative estimate of the salmonellosis risk associated with particular exposures, including that of sheep meat consumption.

These results will be combined with disease surveillance figures, adjusted for under-presentation, under-investigation and under-notification, to estimate the incidence of salmonellosis and *S.* Brandenburg infection attributable to each of the risk factors identified.

1.5 Other projects

A number of research projects on various aspects of *S.* Brandenburg at the farm and processing level have been undertaken by groups outside the *Salmonella* risk assessment project. The data from these studies may be used in the risk assessment depending on their applicability. The reports (Appendix 1) are presented as provided by the authors, although abstracts or summaries have been used for reasons of space. For further information please contact the author(s) cited.

Part II: Risk Management Goals

This section compares the results obtained to date with the outcomes expected by the various stakeholder groups involved in the project, both in terms of overall goals and what was considered the highest priority short-term data needs. The section finishes with the conclusion that the short-term goals have been met and a brief outline of the major areas where work is planned to complete the overall project.

The expectation was that the data gathered in the short-term could be used to manage the risk of *S*. Brandenburg at the level of the farm and processing plant, prior to the completion of the quantitative risk assessment itself. The potential for risk management measures that could be applied both on the farm and in the processing plants were discussed at technical and public meetings held in Invermay in May 2002. However, participants in these meetings supported dissemination of the study results over the recommendation of specific measures, until further information is gained from planned future work.

2.1   Overall project goals

The risk management goals identified at the Gore meeting are discussed below. Goals shared by the stakeholder groups involved are followed by specific goals for each stakeholder group.

Shared goals

- Establish an estimate of occupational risk, and identify optimal risk reduction measures

This goal is being addressed by the case-control study currently being undertaken by ESR. From the human disease data gathered by routine surveillance, it is clear that the farming sector is at risk from on-farm contact with aborted animals. Public health officials, veterinarians and animal health laboratories in affected areas have been active in promoting measures to reduce exposure of farmers and their families (and animals) to the disease, and the number of human (and animal) cases has been declining. These measures are displayed on the NZ Food Safety Authority website at http://www.nzfsa.govt.nz/consumers/food-safety/salmonella-brandenburg/index.htm

- Establish an estimate of food-borne risk, and where appropriate, identify specific risk reduction measures that ensure an agreed level of consumer protection

- Provide strategies and interventions to reduce the potential for adverse overseas market access events arising from the presence of *Salmonella* in fresh meat and meat products

- Provide a model for investigation of other food-borne pathogens.

These latter three goals will be addressed by the completion of the quantitative risk assessment model. Activities to gather the data needed to complete the model are noted later in this document. Further planned work looking at the reason for the drop-off in prevalence over the course of processing may also provide information on possible interventions to reduce *Salmonella* in meat and meat products.

Primary producers (farmers)

- utilise data generated from the on-farm module to prevent and reduce animal health problems in sheep and associated livestock groups

Results from the on-farm studies to date have been made available to farmers to help meet this goal. The most significant results are the association of strip grazing with increased odds of disease. The planned on-farm case-control study beginning in 2002 will help clarify the most important components of this practice on the odds of disease, prior to the recommendation of specific measures.

Processing industry

- develop a case for the "equivalence" of sanitary measures applied to meat and meat products exported from New Zealand

This goal will be addressed by the completion of the quantitative risk assessment model, although the prevalence data collected from meat products will be useful in the development of a case for equivalence, should this be required.

- investigate any animal welfare issues that may be associated with *Salmonella* infection in slaughter populations

This goal has not been addressed to date.

Ministry of Health

- contribute to a risk profile for establishing broad food safety priorities for New Zealand consumers

The results to date from sampling studies and surveillance data indicate that *Salmonella* in sheep meat is unlikely to present a significant risk to the consumer, and this information will be utilised by MOH in setting their food safety priorities. Completion of the risk assessment model will further contribute to this goal.

MAF Food

- ensure optimal market access for sheep meat exported from New Zealand, and develop a case for the "equivalence" of sanitary measures

- utilise the QRA model to optimise existing generic HACCP plans for controlling faecal contamination of fresh meat (all slaughter species)

These goals will be addressed by the completion of the risk assessment model, although a case for equivalence could be made utilising sampling results to date.

- contribute to sustainable agricultural production systems in New Zealand.

This goal is being met as more information is gathered under the wider project, particularly within the on-farm component.

Field veterinarians and the animal remedies industry

- develop cost-effective and efficient controls at the farm level, including vaccines

The on-farm studies to date and those planned will help meet this goal. Pharmaceutical companies are progressing vaccination options (see 1.5 Other projects).

Science providers

- provide an effective scientific contribution to achieving all risk management goals and develop a generic risk assessment capability.

AgResearch, Massey University and ESR have been the major science providers contributing to the success of the project to date.

2.2   Short-term goals

In addition to the overall goals above, participants at the Gore meeting outlined a number of short term data needs, which were considered the highest priority in terms of managing the impact of *S*. Brandenburg. These are listed and assessed under their module headings below, with some discussion on the implications for risk management measures.

On farm module

- Investigation of the *S*. Brandenburg status of ewes aborting in the 2000 lambing season in the south of the South Island.

- Sampling of fleece and faeces from different groups of lambs at the time of initial drafting prior to slaughter

- Survey of "infected" and "non-infected" farms and their relative contribution to pathogen loads entering the slaughterhouse.

- Retrospective mail survey (or personal interview) of selected farms to obtain data on farm, flock and management factors to evaluate associations with the occurrence of *S*. Brandenburg outbreaks

- Monitoring of the impact of any interventions applied at the farm level in the short term should be co-ordinated between the farm module and the processing module.

The first three of these goals were combined into the first on-farm study "*Salmonella* prevalence in lambs and ewes" conducted in association with the first processing study "Prevalence and concentration of *Salmonella* on pelted carcasses". These studies used a case and control methodology to examine the difference in faecal and carcass isolation rates for lambs and ewes from farms known to have experienced *S*. Brandenburg abortions in 2000 and those that had never experienced a case. Fleece samples weren't taken (except for control lambs in February) due to the additional cost compared to the perceived benefit at the time.

The results indicate that although there may be a large difference in faecal carriage between farms that have had *S*. Brandenburg and those that have not, cross contamination can occur at some point in the transport, lairage or processing of stock. Although more work is required to investigate this issue more thoroughly, limiting cross-contamination is a potential means to control carcass contamination rates. However the negative and low isolation rates from boning room samples suggest that efforts to identify the basis for this reduction may give rise to a more effective control measure than trying to reduce contamination of carcasses at the processing stage.

The mail out survey came up with robust results that indicated strip grazing (in particular with back fencing) to be significant risk factors for the occurrence of the disease on-farm. As outlined earlier in this document this is the subject of ongoing study, the results of which should assist local farmers in reviewing at least certain aspects of this practice. Until this study is completed, specific interventions cannot be recommended.

Therefore, monitoring of the effect of interventions on farm will not be done until more information is available on the risk factors and interventions that may have an effect on disease incidence. Monitoring the changing incidence of the disease in the Otago/Southland endemic area has however been introduced from the 2001 season onwards. This is in the form of a questionnaire that will be sent to farmers supplying stock to one of the processing plants in the endemic area, over a certain period on a yearly basis. This will provide a better indication of the changing nature of the epidemic than the use of surveillance data alone.

Processing module

In addition to the above survey of case and control farm animals entering the slaughterhouse, research on the impact on hazard levels of different steps during processing was identified as a short-term priority. Particular steps identified for investigation were:

- Swim washing

- Final carcass washing

- Neck-stringing of carcasses

Swim washing was the subject of recent research carried out by AgResearch, and it was therefore decided not necessary to repeat this work. Results of this research indicate that swim washing is an important contributor to cross-contamination of fleece. The processing industry is working to implement changes, based on these results, outside the framework of this project. Data from this study may be utilised in the quantitative risk assessment model.

Final carcass washing was dropped from the study program due to funds being redirected to the sampling trials. The sampling results taken from carcasses at the end of processing (on the marshalling floor) include the effect of the final carcass wash on levels seen during pelting. This and the other steps between pelting and the marshalling floor can be considered as a group until research on the effect of washing alone can be funded.

Neck-stringing of carcasses. A study commissioned by the Animal Products group of MAF Food, independent to this project, indicated that there was no difference in growth of *E.coli* (surrogate for *Salmonella*), APC (Aerobic Plate Count) and Coliforms between carcasses that were neck strung and those that weren't.

2.3   Risk management goals - conclusions

The short-term goals outlined in the Gore meeting have largely been met, with research conducted both under the project framework and by other interested parties. The intention expressed at that meeting was that industry would use the results to develop codes of practice for primary production, transport, and slaughter and dressing of sheep, with the work co-ordinated by the technical group set up under the project. The utilisation of the results was discussed at technical and public meetings held in Invermay in May 2002, where it was concluded that it would be premature to consider codes of practice. These will be reconsidered as further information is gathered during or as a result of the completion of the quantitative risk assessment model.

To complete the overall goals of the project, the quantitative risk assessment model needs to be completed and any animal welfare issues that may be associated with *Salmonella* infection in slaughter populations investigated.

Completion of the risk assessment model will require the collection of more data, particularly in the processing, storage/distribution/retail and consumer modules. The collection of sampling data at various points in the farm to consumer food chain provide "reality checks" when developing the model, but further explanation of the effect of all the steps in the process is required. Much of this information may be gathered through published literature or even expert opinion but there are a number of key knowledge gaps that are being actively pursued, in particular through planned research projects aiming to clarify:

1)   The distribution pattern of organisms on a carcass at the end of processing, so results from the sampled areas can be extrapolated to meat cuts taken from anywhere on the carcass.
2)   The effect on that distribution of cross contamination by hands, knives, other carcasses etc during processing.
3)   The diversity in practices (that might affect *Salmonella* levels) between processing plants
4)   The reason for the large drop in *Salmonella* isolates from the end of processing to the boning room.
5)   The pH and water activity of the carcasses during the chilling process, including a methodology for measuring water activity on a carcass.
6)   The multitude of pathways meat from a carcass can follow, and what happens to it on its journey to the consumer's table.

The quantitative risk assessment model is expected to be completed in mid-late 2003.

Acknowledgements

Appendix 1 – Other projects

1. Faecal excretion rate in ewes and the carrier state of black-backed gulls (*Larus dominicanus*)

Gary Clark, Susan Swaney (LABNET Invermay), Carolyn Nicol (ESR Communicable Disease Group), Stan Fenwick and Megan Leyland - A MAF Policy funded project.

This project was designed to obtain information on faecal carrier state in ewes and the role of black-backed gulls in the spread of the disease.

Two farms, a farm where *S*. Brandenburg had been confirmed as the cause of abortions and a farm which had not had *S*. Brandenburg, were selected from each of the following areas: Darfield, Ashburton, Milton, Winton and Otautau. In September, on the *S*. Brandenburg farms from the group of ewes that had aborted and on control farms, 20 ewes were tagged, identified and faecal sampled.

On all farms efforts were made to obtain 10 black-backed gulls by poisoning, using alphachloralose (Brown and Keedwell, 1998). In addition to the gulls, 5 ducks were also submitted for sampling.   The 20 identified ewes from each farm were resampled when lambs were weaned, in March and in June/July period.

The ewe faecal samples and intestinal samples from the hind-gut of the black-backed gulls were tested for *Salmonella* using a selective media method.

Nine black-backed gulls from which a *Salmonella* group B serotype was isolated were further sampled and a total count performed.

All positive isolates were sent to ESR CDG for serotyping.   *S*. Brandenburg isolates also had their molecular pattern typed using pulsed field electrophoresis.

Results

Table 1   *Salmonella* Brandenburg isolations from sheep faeces and black-backed gull intestinal contents on farms where *S* Brandenburg occurred and farms where it was not diagnosed

Farms where *S* Brandenburg abortions had occurred								
District	Number of positive ewes				Number of positive gulls			
	Sept	Dec	Mar	Jun/Jul	Sept	Dec	Mar	Jun/Jul
Darfield			0/20	0/16~	0/4			
Ashburton	9/20	0/20	0/20 @*	0/18	3/7 2/7 1/5 ducks			
Milton	4/20	0/19	0/19	0/20	1/10			0/10^
Winton	9/20	2/19	1/20	0/21	7/9		0/2	
Otautau	7/20	0/19	1/17	0/19	none sampled			
Average	36%	3%	2%	0%	37%	29%	0%	0%

Farms which *S*. Brandenburg was not diagnosed								
Darfield			0/20	0/20~	3/11			
Ashburton	0/20	0/16	0/20 #	0/21	0/1			
Milton	0/20		0/20	0/20	7/10	1/10	0/8 ducks	0/6 rocks
Winton	0/20	0/19	0/20	0/20	3/ 5			
Otautau	0/20	3/11	0/20	0/29				1/3$
Average	0%	7%	0%	0%	52%	10%	0%	33%

@   Same property different sheep
~   Sampled in August
*   3/20 with *Salmonella* Typhimurium
#   1/20 with *Salmonella* Enteritidis
^   1/10 *Salmonella* Hindmarsh
$ Sampled July 29 & 30 1999
Rocks   faecal contaminated from black-backed gull nesting area

Discussion

In September, farms which had *S*. Brandenburg abortions had the organism isolated from 33% of the ewes sampled but no *salmonella* was isolated from ewes faecal sampled from the control farms. This suggests that the control farms were not greatly contaminated with *S*. Brandenburg but the numbers sampled were too small to say there were no infected sheep on the farm.

On one of the farms that had no abortions due to *S*. Brandenburg, three of 11 ewes sampled were excreting the organism at weaning in early February, yet no sheep had been brought onto the farm. The farm was about 10 kilometres away from the nearest confirmed case and the farmer considered black-backed gulls were not present in any significant numbers. The sheep were not vaccinated against *Salmonella* and there was no evidence of an abortion outbreak or deaths in ewes over lambing in 1999. Three black-backed gulls were killed in late July and one had low numbers of *S*. Brandenburg in its intestinal contents. It may be that *S*. Brandenburg infection found in the ewes in the summer occurred via black-backed gulls, but it is interesting that clinical disease did not occur then or in late pregnancy. This probably reflects the importance of other factors, including stress, in initiating clinical disease.

The isolation of *S*. Brandenburg from about 42% of black-backed gulls from *S*. Brandenburg abortion farms and 52% from control farms and the high total counts, up to 25 million organism per gram of gut contents, indicates these birds had the potential to spread the disease.

Black-backed gulls are scavengers, which commonly ingest placenta and tissue from dead foetuses. They are also reported to carry *Salmonella* species in their intestinal tracts with out any obvious affects to themselves. The total counts found being related to the numbers of organisms present in the ingested material. Large numbers of organisms were present in

sheep placenta and foetal tissues examined at the laboratory. Histological examinations of samples frequently showed capillaries packed with the organisms. Therefore, the high total counts found in the gulls were to be expected.

The presence of *S*. Brandenburg in a duck is of interest. Ducks do not scavenge dead foetuses or placenta. Therefore, the organism was probably picked up from contaminated pasture or water and thus reflects environmental contamination of the organism.

References

Clark G., Fenwick S., Boxall, N, Swanney S, Nicol C. *Salmonella* Brandenburg abortions in sheep, pathogenesis and pathology. Society of Sheep & Beef Cattle Veterinarians, New Zealand Veterinary Association Annual Seminar, 12-22, 1999.

Clark G, Swanney S, Nicol C. Fenwick S. *Salmonella* Brandenburg – the 1999 season. Proceedings Sheep and Beef Cattle Society of Veterinarians New Zealand Veterinary Association, Palmerston North, pp 151-157, 2000.

2.   Spread of *Salmonella* Brandenburg organisms in sheep yards

Gary Clark, Susan Swaney (LABNET Invermay), Carolyn Nicol (ESR Communicable Disease Group), PD Johnstone (AgReseach, Invermay), Stan Fenwick and Megan Leyland   - A MAF Policy funded project

A likely cause of spread in flocks is by inhalation of salmonella-contaminated dust in sheep yards. Sheep are more susceptible to infection of salmonellae by inhalation than by ingestion of the organism. Ewes have been shown to excrete salmonellae rapidly following normal yarding[1].

In a study involving 141 farms in the North Island, 66 farms had ewes dying from salmonellosis (*S*. Typhimurium and *S*. Bovis-morbificans). On 58 of these farms salmonellae were isolated from samples of soil and surface debris from yard environments[2].

This study set out to compare the prevalence of *S*. Brandenburg in the dust of sheep yards of farms where *S*. Brandenburg abortions have occurred, and those where no abortions are thought to have occurred.

Sheep yard dust samples were collected in January, March, June-July period and mid-October 2000, after lambing, from 32 *S*. Brandenburg "1999 affected" farms and 32 "1999 control" farms. In October, one "1999 control" farm did not participate as the sheep yards had been converted into a calf-rearing unit and the area covered in 30 cm of sawdust.

At least 50 gram samples of sheep yard dust were collected mostly from the holding pens close to the drafting race.

Samples were cultured for *Salmonella* using MIRINZ method and positive isolates sent to ESR CDG for serotyping. *S*. Brandenburg isolates from 19 farms had their molecular pattern typed using pulsed field electrophoresis.

Information was also collected on whether yards had been cleaned and disinfected since last sampling, whether abortions had occurred this season (2000) and if they had whether this had been confirmed as *S*. Brandenburg by laboratory tests and whether or not ewes had been vaccinated against *S*. Brandenburg.

Results

Figure 1   *Salmonella* species isolated from sheep yard dust samples in 2000 from *Salmonella* Brandenburg affected farms and non-affected farms
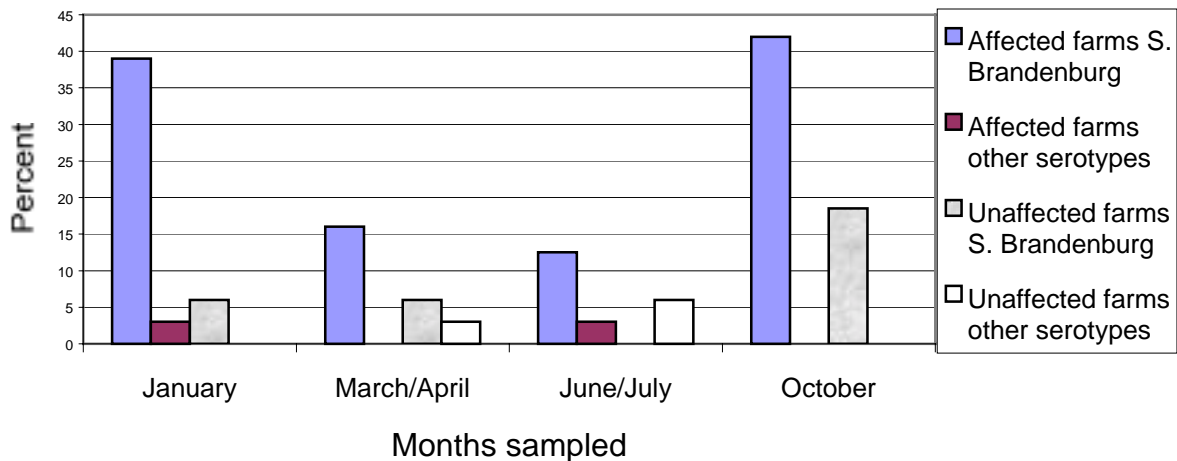
There was an increased number of farms with *S*. Brandenburg cultured from their sheep yard dust in October compared with January, March/April and June/July sampling periods.

Four (13%) of the original unaffected farms had *S*. Brandenburg abortions, laboratory confirmed, with one other farm having abortions suspected to be *S*. Brandenburg, but no laboratory confirmation as to cause.   Based on confirmed cases there were 36 affected farms and *S*. Brandenburg was isolated from sheep yard dust on 15 (43%) of these farms. Of the remaining 27 farms with no confirmed *S*. Brandenburg abortions five farms (18.5%) had *S*. Brandenburg cultured from the sheep yard dust

Of 43 farms that had evidence of the organism on the farm 72% had vaccinated all or part of their ewe flock using Salvexin + B or Salvexin. Abortions confirmed or thought to have been due to *S*. Brandenburg occurred to a greater degree (2.56 times odd ratio) on unvaccinated "affected farms" compared to vaccinated "affected" farms.

In October, one of the *S*. Brandenburg isolates from a "1999 affected" farm had a different pulse-field electrophoretic DNA pattern, involving a loss of one band and the addition of three bands of lower molecular weight.

Discussion

The increased number of farms with *S*. Brandenburg cultured from their sheep yard dust in October, compared to earlier in the year, is compatible with increased environmental contamination of the organism during the abortion/lambing period, and post lambing when the yards are used for handling ewes and lambs. This is likely to be due to faecal excretion, as a previous study found that 36% of ewes from groups affected by abortions were excreting

S. Brandenburg in their faeces in September. Lambs may also be a source of faecal excretion. Other sources of infection can be ewes aborting in the yards and or uterine discharges from recently aborted ewes that have been brought into the yards.   Birds carrying the organism may be an additional source of infection.

The change in the *S*. Brandenburg DNA pattern is of interest in relation to possible change in pathogenicity and also spread of this isolate on this farm and to other farms.

Of the 43 farms that had evidence of the organism on the farm in 1999 or 2000, 72% had vaccinated all or part of their ewe flock using Salvexin + B or Salvexin. There appeared to be higher odds on getting *S*. Brandenburg abortions in non-vaccinated flocks, versus vaccinated flocks, on farms known to have the organism present, but the number of farms in the study were too small to show a significant effect.   Although vaccination is not 100% protective, it has been shown that vaccination with Salvexin does give some significant protection from the disease[3].

References

1.   Robinson RA. *Salmonella* excretion by sheep following yarding. New Zealand Veterinary Journal, 15, 24-25, 1967.

2.   Robinson RA, Royal WA. Field epizootiology of S*almonella* infection in sheep. New Zealand Journal of Agricultural Research, 14, 442-56, 1971.

3.   Marchant, R. *Salmonella* Brandenburg disease in sheep: Vaccine development and experiences. 30th Seminar of Society of Sheep & Beef Cattle Veterinarians, New Zealand Veterinary Association Annual Seminar, 159-166, 2000.

3.   Spread of *Salmonella* organisms in sheep yards and effect on lambs and ewes at meat plants

Gary Clark, Susan Swaney (LABNET Invermay), Carolyn Nicol (ESR Communicable Disease Group), PD Johnstone (AgReseach, Invermay), Stan Fenwick and Megan Leyland   - A MAF Policy funded project

A likely cause of spread in flocks is by inhalation of salmonella-contaminated dust in sheep yards. Sheep are more susceptible to infection of salmonellae by inhalation than by ingestion of the organism. Ewes have been shown to excrete salmonellae rapidly following normal yarding[1].

Although *S*. Typhimurium and *S*. Hindmarsh can spread in this manner it is not known what role yards may have in the spread of *S*. Brandenburg.   This study was aimed at determining the likely role of sheep yards in the spread of *S*. Brandenburg in sheep.

Ten lambs and 10 ewes from 10 control farms, where no *S*. Brandenburg had been isolated from sheep yard dust, were kept for 4 to 6 hour in yards from three farms where *S*. Brandenburg was isolated from sheep yard dust in late January.   These sheep were slaughtered 24 hours later at the meat plant and two or more mesenteric lymph nodes were collected from each and sent to the laboratory for culture, using routine selective media.

All *Salmonella* positive isolates were serotyped at ESR Communicable Disease Group and if identified as *S*. Brandenburg were tested for molecular pattern using pulsed-field gel electrophoresis.

Results

The attempt to infect ewes (10) and lambs (10) from each of 10 control farms by running them through an *S*. Brandenburg-affected sheep yard for about 4 to 5 hours was unsuccessful.

Discussion

Although the organisms in the dust could be a source of infection for sheep being yarded this was not proven in the trials above. This may have been due to lack of organisms, as *Salmonella* could not be isolated from sheep yard dust at the time of the trial from two of the three affected farms, and or the sheep spent insufficient time in the affected yards.

It does reinforce a recommendation that ewes should be yarded for a minimum of time. This is to reduce the risk of picking up an infection in the yards and to minimise a feed check, which can increase numbers of *Salmonella* in the gut.

References

1.   Robinson RA. *Salmonella* excretion by sheep following yarding. New Zealand Veterinary Journal, 15, 24-25, 1967.

4.   *Salmonella* Brandenburg – a new molecular pattern its occurrence on the farm of origin and a study on possible spread

Gary Clark[*], Susan Swanney[*], Carolyn Nicol[#], Megan Leyland [@]

The emergence of a new strain of *S*. Brandenburg in a previous study[1] gave an opportunity to investigation the possible spread of the new strain and if it showed a change in pathogenicity.

The study involved the index farm, four neighbouring farms and farms that had introduced rams from the index farm. The latter farms were greater than 50 kilometers away. This distance was chosen to minimise the risk of infection spreading from the index farm to these farms by black-backed gulls contaminating pasture or stock water. Black-backed gulls generally feed within 50 kilometers of their nest and mostly within a lesser radius.

Farmers participating in the investigation were asked to submit aborted foetuses to the laboratory. Samples were collected from aborted foetuses and cultured for *Salmonella.*

Sheep yard dust samples were also collected post lambing, in October, from all these farms and cultured for *Salmonella* using the MIRINZ method (Reid and Cook, 1991).

Salmonella isolates were sent to ESR CDG for serotyping. The molecular pattern of *S*. Brandenburg isolates was determined using pulse field gel electrophoresis (Wright et al, 1996).

Results

S. Brandenburg abortions occurred on the index farm and the organism was isolated from four foetuses. Three of four neighbouring farms had abortions and one of these farms had *S.* Brandenburg isolated from seven foetuses. An additional farm, neighbouring this one and further down stream also had *S.* Brandenburg isolated from two aborted foetuses.

A farm over 50 kilometers from the index farm that had brought rams from the index farm had *S.* Brandenburg isolated from two aborted foetuses.

S. Brandenburg was isolated from sheep yard dust samples from the index farm (seven sub-samples), two neighbouring farms (seven sub-samples) and four of the seven farms over 50 kilometers from the index farm. All the farms that had *S.* Brandenburg sheep abortions had the organism isolated from the sheep yard dust. Of nine farms that had no evidence of *S.*Brandenburg abortions four had *S.*Brandenburg isolated from sheep yard dust.

On PFGE testing, the *S.* Brandenburg isolates all had the same endemic field strain molecular pattern. There was no evidence of the new strain.

Discussion

The occurrence of *S.* Brandenburg abortions on the index farm, a neighbouring farm and near neighbour plus isolation of *S.* Brandenburg from sheep yard dust on the index farm and two neighbouring farms gave the opportunity for the new strain to be detected if it was going to increase and spread. The failure to isolate the new strain suggests it may be less viable and or less virulent than the endemic strain. However, more PFGE testing of sheep yard dust and animal isolates on the index farm is required to substantiate this hypothesis.

Once again *S.* Brandenburg has been isolated from sheep yard dust samples on farms where no clinical evidence of disease has occurred. This occurred on four of nine farms. In a previous study, 18.5% of farms that had no laboratory confirmed evidence of *S.* Brandenburg sheep abortions had the organism isolated from sheep yard dust[1]. This supports previous findings that the bacteria can be present on farms without evidence of clinical disease and means that care is required by farm workers and families to minimise the risk of contacting the disease, especially on farms within the endemic area.

References

1. Gary Clark, Susan Swaney (LABNET Invermay), Carolyn Nicol (ESR Communicable Disease Group), Stan Fenwick and Megan Leyland. Spread of *Salmonella* Brandenburg organisms in sheep yards. MAF Policy funded project.

Acknowledgements

5. The use of an attenuated *Salmonella Typhimurium* vaccine in sheep to prevent abortion due to *Salmonella Brandenburg*: challenge and field trials.

Christensen NH[1], McFarlane RG[2], Robinson RA[3], Sherwood D[4], Greenlees R[5], Wagner JC[6] and Deitemeyer KW[7].

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Salmonella Brandenburg is a group B salmonella with the antigenic make-up, **O:**1,4,12; **H1**: 1,v; **H2**: e,n,z_{15}. The registration (ARB 7935) of Meganvac-1, a live, attenuated *(Δcya, Δcrp) Salmonella Typhimurium* vaccine for control of salmonella infections of public health significance in poultry provided a possible means of control of the SB-disease in sheep, due to the cross immunity to other group B salmonellae. The advantage of using such live, attenuated salmonellae as vaccines is that they stimulate humoral (serum antibodies), secretory(antibodies active on mucosal surfaces) and cellular immune (killer cells in blood and tissues) responses following oral administration, while killed vaccines, which must be injected, tend to stimulate strong antibody production only. Safety trials were successfully completed in 2000 and were reported elsewhere (Khalil *et al* 2001). The vaccine was then administered intra-occularly (to take advantage of the antigen –processing and presenting capabilities of the Harderian gland) to pregnant ewes followed by a controlled challenge via natural routes of infection with virulent SB. The safety of the vaccine was also confirmed by a field trail in which 3500 pregnant hoggets, two tooth and older ewes were vaccinated soon after mating on farms with a history of *Salmonella Brandenburg* infection. This trial took place in the 2001 lambing season and the farms used experienced no evidence of SB challenge.

Results
Summary results of the challenge trial are shown below in Table 1

Table 1: Results of challenge of ewes with *Salmonella Brandenburg*

Treatment group	No. ewes	SB pos faecal samples 48 hr	Ewes not producing live lambs	No. ewe deaths SB related	other causes	% SB ewe deaths	ewes lambed	No. lambs
LD SB Unvaccinated	7	4	0	0	0	0%	7	10
HD SB Unvaccinated	8	8	3	2	1	25%	5	6
HD SB Vaccinated Eye drop	8	5	2	0	0	0%	6	8
HD SB Vaccinated Orally	7	5	3	2	0	29%	4	6
Unvaccinated unchallenged	1	0	0	0	0	0%	1	1

HD = high dose SB LD = low dose

Five ewes died or were euthansed in moribund condition during the trial. Four of these deaths showed signs typical of *Salmonella Brandenburg* infection. In addition to the five ewes that died, a further 3 ewes aborted and survived. Aborted foetuses were severely autolysed. The results of bacteriological examination of the aborted foetuses and ewes are shown in table 2.

Table 2: Results of microbiological examination of ewes not producing live lambs, and aborted foetuses.

Ewe No.	9	10	16	22	24	27	32	34
Treatment	HD SB Unvacc	HD SB Unvacc	HDSB Unvacc	HD SB Vacc Eye	HD SB Vacc Oral	HD SB Vacc Oral	HD SB Vacc Eye	HD SB Vacc Oral
SB Faecal result 48 hrs post challenge	pos	Pos	pos	Pos	pos	pos	pos	pos
Ewe comment	euthanased sleepy sickness	Aborted died	died	Aborted survived	aborted euthansed	aborted euthansed	aborted survived	aborted survived
Days post challenge of death/euthansia	14	22	20	n/a	32	13	n/a	n/a
Days post challenge of abortion	n/a	20	n/a	14	30	13	12	14
SB foetal culture	neg	Pos	pos	Pos	neg	pos	n/t	pos
Ewe rib	neg	Pos	pos	n/a	neg	pos	n/t	n/t
Other organs					faeces pos SB			

n/a not applicable   n/t not attempted

Detailed results from all 31 ewes are provided in table 3

Table 3: *Salmonella Brandenburg* isolated from faecal samples at various times before and after vaccination and challenge

Treatment group	No. ewes	*Salmonella Brandenburg* isolated from faecal samples			
		pre vaccination	24 hrs post vacc 1	24 hrs post vacc 2	48 hrs post challenge
LD SB Unvaccinated	7	0	Nt	nt	4
HD SB Unvaccinated	8	0	Nt	nt	8
HD SB Vaccinated Eye drop	8	0	0	0	5
HD SB Vaccinated orally	7	0	0	0	5
Unvaccinated unchallenged	1	0	nt	nt	nt

Survival of *Salmonella Brandenburg* in yard dust

Salmonella Brandenburg was detected in yard dust from 24 hours after deposition, through the very dry period through to 18 days post challenge, and then after the severe frosts of the 2001 winter to 65 days post challenge, but not in a sample collected 209 days post contamination. The results are shown in table 4.

Table 4: Survival of *Salmonella Brandenburg* in yard dust

Days post contamination	-1	1	18	35	65	209
Result of SB culture	neg	pos	pos	pos	pos	neg

Discussion

The challenge

The reproduction of abortion in sheep by artificial infection with salmonellae is a hit and miss process with variable results reported. Abortion in pregnant ewes was successfully reproduced by oral dosing with *Salmonella Montivideo* (Linklater 1983), but Taylor *et al* (2001) were unable to reproduce abortion using intranasal *and* oral inoculation of pregnant ewes with 1 ml

of a 1 x 10^9 CFU/ml of *Salmonella Brandenburg* culture. Although the ewes in that trial shed organisms in their faeces, no abortions occurred and the authors had to resort to the somewhat artificial means of intravenous challenge to induce abortion. Although injection of 1 x 10^7 CFU/ml resulted in abortion in a single animal, the trial ewes were reliably induced to abort only at a dose of 1 x 10^9 CFU/ml. Within 2 days, abortions had commenced and, by 7 days post-challenge, all of the ewes had aborted. Prior to abortion, animals were seen to be lethargic, anorexic and feverish. This is a more concentrated abortion storm than the events described in the present trial, presumably due to the unnatural route of infection.

Table 2 provides some valuable insights into SB abortion under reasonably natural challenge conditions:

- All aborting ewes shed SB in the faeces 48 hours post challenge
- The time from challenge to abortion/death varied from 12 to 32 days, with a median period of 14 days.
- SB was recoverable from foetus or rib in all but one case – the one that survived for 32 days

A list of references is available from the author

6. Experimental infection of pregnant sheep with attenuated *Salmonella typhimurium*

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ABSTRACT

Salmonellosis occurs in many animal species including sheep, and may cause pathology of the gut and possible abortion. If attenuated strains were to be used for future control, their establishment in the host and shedding pattern needs to be determined. Fifty-one pregnant ewes were obtained from the Lincoln University Research Farm and assigned randomly to five treatment groups. At approximately 1 month before lambing, the groups were given attenuated *S. typhimurium* (*cya*⁻, *crp*⁻) by different routes and doses as follows: 10^{10} colony-forming units (cfu) intranasally (i.n.), 10^9 cfu i.n., 10^{10} cfu subcutaneously (s.c.), 10^9 cfu s.c., and control (saline i.n. and s.c.). Selected animals were slaughtered at days 4 and 14 post infection for bacteriological culture from the mesenteric and retropharyngeal lymph nodes, intestinal contents and lamb stomach contents. The experimental organisms were isolated using selective cultures and identified by latex agglutination test and fermentation reactions. Following infection there were no changes in rectal temperature or significant clinical signs except two cases of abortion in the 10^{10} cfu s.c. group. The number of animals shedding organisms in their faeces decreased with time from 12 ewes at day 1 to one ewe at day 4 and day 7. Most of them were from the 10^{10} i.n. group. Subcutaneous injection of 10^{10} cfu induced abortion in two of 11 ewes, as organisms were detected in their faeces and the aborted lambs. However, no organisms were detected in faeces, intestinal contents or the lamb stomach contents of the ewes that were killed at days 4 or 14. In the two ewes that had received 10^{10} cfu i.n. and were killed at day 4, organisms were detected in the mesenteric and retropharyngeal lymph nodes. In summary, the administration of the attenuated *S. typhimurium* (*cya*⁻, *crp*⁻) at 10^{10} cfu caused asymptomatic short-term faecal shedding and abortion when given i.n. and s.c., respectively.

7.   SALVEXIN ® +B SHEEP VACCINATION

SURVEY 2000

SUMMARY POINTS:

- Vaccination of 2 tooths and mixed age ewes reduced the incidence of abortions and deaths when disease occurred.

- In vaccinated 2 tooths abortion incidence was reduced by 59% and the incidence of deaths in aborted sheep by 74%.

- Unvaccinated 2 tooths are 2.6 times more likely to abort and 1.5 times more likely to die than vaccinated 2 tooths.

- In mixed age ewes the abortion incidence was reduced by 65 and 54%, and the incidence of deaths was reduced by 73 and 64% when ewes were given 1 or 2 vaccinations respectively.

- Unvaccinated ewes are 3.0 and 2.3 times more likely to abort and 1.6 times more likely to die when disease occurs than ewes given 1 or 2 vaccinations.

- On an average 500 2 tooth and 2000 mixed age ewe farm, vaccination gives an economic benefit of $2,300 in the 2 tooths and $10,700 and $7,500 in ewes given 1 or 2 vaccinations.

- Unvaccinated sheep, both 2 tooths and mixed age ewes are at greater risk where there has been no Brandenburg disease the previous season.

NOTE:

Because of the severe nature of Brandenburg disease vaccination will not always give 100% protection, however when disease does occur in vaccinated mobs they will be better off than if they had not been vaccinated.