THE PREVALENCE AND PUBLIC HEALTH IMPLICATIONS OF *Salmonella brandenburg*, AN EMERGING PATHOGEN OF SHEEP IN NEW ZEALAND

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DEDICATED TO THE MEMORY OF OUR LATE SISTER, KGALALELO

ABSTRACT

Since the first case of *Salmonella brandenburg* abortion was recorded from a single sheep farm in the South Island of New Zealand in 1996, the outbreak had spread to more than 300 farms by the lambing season of 2000. This study, which was funded by Meat New Zealand and other stakeholders, is a pilot project to estimate the prevalence of *Salmonella brandenburg* and consider its potential foodborne and occupational health risk implications.

Eight farms, four control farms and four affected farms were chosen from known infected areas. Control farms were those which had not experienced *Salmonella brandenburg* outbreaks, while the four affected properties had clinical outbreaks during the 2000 lambing season which had either been laboratory confirmed or not. At each farm faecal samples were collected from 50 lambs and 50 ewes at drafting and at slaughter. Therefore a total of 200 samples were taken from each group of animals sent for slaughter. The sampling was done in two phases to determine the effect of time interval on the prevalence of *Salmonella brandenburg* in sheep between abortion outbreaks and slaughter. The same sampling routine was followed for the November-December (Phase I) and February-March (Phase II) periods. The isolates from faecal cultures that had been confirmed as *Salmonella* spp. by slide agglutination test were sent to the Institute of Environmental Science and Research (ESR) for serotyping. All the 133 samples sent for serotyping turned out as *S. brandenburg.*

The primary comparison of the study was done between the prevalence of *Salmonella brandenburg* in animals from affected farms and control farms. In addition the study was also interested in comparing *S. brandenburg* prevalence **within** class i.e. in lambs or ewes from the same farm at drafting and at slaughter. Comparison was also made **between** classes i.e. the prevalence of the organism in lambs and ewes from the same farm at drafting and at slaughter.

During phase I of the on-farm sampling the prevalence of *Salmonella brandenburg* in tested lambs and ewes from affected farms was 12.0.% and 18.7% respectively. The on-farm prevalence for the control farms was 4.0% for lambs and 3.5% for ewes. During phase I of abattoir sampling the overall prevalence for the affected farms was 9.0 % for lambs and 22.0 % for ewes compared to 0.0% for lambs and 1.0% for ewes from control farms. The high prevalence of *Salmonella brandenburg* in animals from affected farms as compared to control farms showed that affected farms were associated with high excretion rates and therefore high levels of environmental contamination.

During phase II of the on-farm sampling the prevalence of *S. brandenburg* in tested lambs and ewes from affected farms was 2.5% and 2.7% respectively. The phase II on-farm prevalence of *S. brandenburg* from control farms was 0.0% for lambs and 0.8% for ewes. During the same phase, abattoir prevalence of *S. brandenburg* in lambs and ewes from affected farms was 0.0% and 2.7% respectively compared to 0.5% for lambs and 0.0% for ewes from control farms. Like in phase I the overall prevalence of *S. brandenburg* was higher in animals from affected farms as compared to animals from

control farms. The study also showed that the prevalence of the organism was very high during phase I compared to phase II irrespective of class of animal or site of sampling. This could have been due to the high number of animals still excreting the organisms closer to the outbreak period or the high level of environmental contamination. Both factors would have contributed to a higher prevalence of positive cultures. The higher prevalence of positive cultures during the November-December period as compared to the February-March period showed that the risk of infection and product contamination was greatest at commencement of the season (November-December) but was greatly reduced by February. Therefore further research is required to find the production and processing methods that might reduce the risk of infection and product contamination during the period of November-December.

However the positive cultures of *Salmonella brandeburg* in control farms suggested a spreading disease outbreak and that the absence of clinical outbreaks of the disease did not mean an absence of infected animals on-farm. It is very important to do further investigations to find on-farm risk factors that might result in the absence or presence of clinical outbreaks.

The study did not show any obvious differences in the *S. brandenburg* prevalence **within** class between on-farm and slaughter samples. There was also no obvious difference in the prevalence of the organism **between** ewes and lambs from the same farm, either during on-farm or abattoir sampling.

Pulsed-field gel electrophoresis (PFGE) of the 24 isolates, which were a representative sample of the study, gave an identical profile. The PFGE and the serotyping suggested that the outbreak strain had become the dominant serotype in the sampled farms in the outbreak regions of the South Island of New Zealand. Therefore factors that gave rise to this dominance should be further investigated.

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LIST OF ABBREVIATIONS

BGA	brilliant green agar
BHI	brain heart infusion
BPW	buffered peptone waters
EDTA	ethylene diamine tetra-acetic acid
ELISA	enzyme linked immunosorbent assay
ESR	Institute of Environmental Science and Research
FAE	follicle associated epithelium
LIA	lysine iron agar
MQ	Milli-Q
MUCAP	4-methylumbel-liferyl caprylate
PCR	polymerase chain reaction
PFC	pulsed-field certified
PFGE	pulsed-field gel electrophoresis
RFLP	restriction fragment length polymorphism
RVS	Rappaport-Vassiliadis
SPI	Salmonella pathogenicity island
TBE	trisbase, boric acid, EDTA
TE	tris-HCl, EDTA
TSA	trypticase soy agar
TSI	triple sugar iron
XLD	xylose lysine desoxycholate

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CHAPTER ONE – GENERAL INTRODUCTION

1.1 Introduction

Historically disease in sheep in New Zealand caused by *Salmonella* spp. was associated with management practices in summer and autumn (from early January to May). These management activities included change in nutrition, transport to slaughter plants, mustering and yarding (Marchant, 1999; Fenwick, 2000). Salmonellosis manifested clinically as diarrhoea and death, with *S. hindmarsh* and *S. typhimurium* the commonly isolated serotypes (Marchant, 1999). Cases of *Salmonella* spp. abortion in sheep in New Zealand were sporadic and usually caused by the serotypes *hindmarsh, typhimurium* and *oranienburg* (Clark *et al.*, 1999). Overseas ovine abortion had been mainly caused by the host adapted *Salmonella abortusovis* and the non-host adapted *Salmonella montevideo* (Marchant, 1999). However, the picture of *Salmonella* spp. abortion in sheep changed in 1996 after the first case of *Salmonella brandenburg* was reported in the South Island. Previously as reported by Hosie (1991) *Campylobacter* spp. and *Toxoplasma gondii* accounted for 42% and 33% respectively of ovine abortions in New Zealand.

Since 1996 there has been an increase in the number of reports of *Salmonella brandenburg* abortions in ewes in late pregnancy in the South Island of New Zealand. The abortion storms were reported to affect at least 30 % of the ewes in affected flocks with up to 100 % mortality in aborting ewes. By the winter of the 2000 lambing season

the abortion storms had occurred in the regions of Canterbury, Otago and Southland (Clark, 1999; Clark 2001b). Since the first case was reported in a merino flock in Canterbury in 1996, the disease has progressed so that by the 2000 lambing season there were 337 farms with laboratory confirmed cases. The cases were from both sheep and cattle farms (Table 1).

Table 1.1: The number sheep and cattle farms with laboratory confirmed cases of *Salmonella brandenburg* infections

Lambing season	Canterbury farms	Otago farms	Southland farms
1996	1 (0)*	0 (0)	0 (0)
1997	17 (0)	0 (0)	1 (1)
1998	31 (3)	55 (2)	67 (0)
1999	45 (5)	71 (4)	162 (10)
2000	36 (4)	62 (16)	233 (40)

* cattle farms in brackets
Table from Clark (2001b)

The economic loss due to abortions, ewe mortality, a possible loss of access to the export markets and public health issues posed by outbreaks of *Salmonella brandenburg* abortion, prompted the stakeholders in the sheep industry to propose a pilot study of the problem. The key aspect of the pilot study was to evaluate the implications of the outbreaks of *S. brandenburg* in ewes on the risk of infection of ewes and lambs at the time of slaughter.

The general hypothesis of the study was that the occurrence of abortion outbreaks was associated with the risk of *Salmonella* spp. contamination of meat at slaughter and that the contamination was highest at the commencement of the slaughter season (November-

December), closer to the abortion outbreaks. The other hypothesis was that the prevalence of *Salmonella* spp. was high in animals at slaughter compared to that of the same animals on farm. This hypothesis was based on the fact that with stress of congregation on farm, transportation and lairage the animals will be induced to excrete the pathogen. Also during drafting, transportation and lairaging the close contact between the animals would facilitate the spread of salmonellae to previously unexposed animals (Robinson 1967; Grau *et al.*, 1968; Grau *et al.*, 1969; Grau & Smith, 1974; Wray *et al.*, 1991; Gough & McEwen, 2000). The prevalence of *Salmonella* spp. in animals on-farm and at slaughter in the pilot study was estimated by culture of rectal faeces collected on farm and culture of caecal contents at slaughter. Descriptive analysis was then applied on the data collected.

Salmonella brandenburg isolates from the study were subtyped using pulsed-field gel electrophoresis (PFGE). This enabled the determination of the genetic relatedness between the isolates on farm and those at the abattoir compared to the original isolates from the 1996 outbreaks. PFGE has been shown to have a higher discriminatory power than other genomic typing methods like IS 200, ribotyping and restriction fragment length polymorphism (RFLP) in the typing of *Salmonellla brandenburg* isolates (Baquar *et al.*, 1994; Olsen *et al.*, 1993). PFGE is based on the embedding of the microbe in agarose, lysis of the embedded microbe, cutting the genome of the lysed microbe with a rare cutter, transferring the agarose embedded DNA fragments into agarose gel wells and separating the fragments using electricity. Since the mid 1980's PFGE has been replacing serotyping as method of choice for microbial typing (Tenover *et al.*, 1995).

1.2 Objectives Of The Research

The specific objective of the study was to evaluate the implications of the outbreaks of *S. brandenburg* disease in ewes on the risk of infection of ewes and lambs at the time of slaughter. This was achieved by comparing the prevalence of *Salmonella brandenburg* in lambs and sheep from case and control farms, on-farm and at slaughter. The animals were sourced from the outbreak area of Southland, in the South Island of New Zealand.

The secondary objectives were to determine:

- The influence of time interval between ewe abortions and slaughter on prevalence of *Salmonella brandenburg* in animals from affected and control farms presented for slaughter
- The influence of class of animal (lambs compared with ewes) on the prevalence of *Salmonella brandenburg* in animals presented for slaughter
- The influence of transport and lairage on the prevalence of *Salmonella brandenburg* in animals at slaughter i.e. the on farm compared with the slaughter prevalence