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***SALMONELLA BRANDENBURG IN NEW ZEALAND SHEEP:
THE DEVELOPMENT OF A SEROLOGICAL DIAGNOSTIC TEST
AND A CASE CONTROL STUDY***

*A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF VETERINARY STUDIES AT MASSEY UNIVERSITY*

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ABSTRACT

Salmonella Brandenburg causes acute diarrhoea and severe illness in a variety of animals and was first isolated in New Zealand in 1986. Since 1996 *Salmonella* Brandenburg has been associated with an emerging epidemic of abortions and deaths in sheep in the southern regions of the South Island. Little is known about the specific epidemiology of *Salmonella* Brandenburg in sheep and as a result control to date has been largely based on anecdotal evidence and general principles. This study focused on the following aims:

- To develop a serological test for use in epidemiological studies and for monitoring future control efforts targeting *Salmonella* Brandenburg in New Zealand sheep.
- To identify factors associated with the occurrence and severity of *Salmonella* Brandenburg outbreaks in New Zealand sheep.

Traditionally *Salmonella* diagnosis has depended on bacteriological culture. Such tests are time consuming, labour and equipment intensive, and may lack sensitivity. ELISA (Enzyme-Linked Immunosorbent Assay) methodologies offer an alternative for the diagnosis of *Salmonella* infection. Therefore the development of an ELISA test for detecting antibodies to *Salmonella* Brandenburg organism in sheep plasma was undertaken. Expression of common antigens has resulted in a high level of antibody cross-reactivity between different serovars in serological tests. Lipopolysaccharides (LPS) (O Antigens) are the primary cause of these cross-reactions. Cross-reactivity with two common sheep serovars (*Salmonella* Typhimurium and *Salmonella* Brandenburg) was of major concern for the development of a *Salmonella* Brandenburg ELISA. This was overcome by preparing an antigen mainly composed of flagella and fimbria proteins (LPS free). The antigen preparation was of a relatively crude and non-characterised nature and could only produce a reasonable optical density response at a high concentration. Unfortunately, while the ELISA was responsive, the specificity of the ELISA for *Salmonella* Brandenburg antibodies remained poor. Further investigation of the specificity of the antigen preparation, through the use of different sera, or through the development of a more pure and specific antigen, is needed for the successful development of a sensitive and specific serological test for determining *Salmonella* Brandenburg exposure in New Zealand sheep.

A case control study was performed as part of a large-scale ongoing investigation aimed at identifying factors associated with *Salmonella* Brandenburg disease in New Zealand sheep. Details of disease prevalence and farm management methods were collected from two affected regions in southern New Zealand. Associations between possible risk factors and *Salmonella* Brandenburg were evaluated using odds ratios, with analyses being performed at two different levels:

- farm level analysis to compare affected vs. unaffected farms using a case-control approach.
- within farm analysis restricted only to affected farms to evaluate risk factors associated with severity of reported disease on affected farms.

Data were collected from 405 farms containing a total of 1, 170,737 ewes. Of the 175 case farms, 97% had diseased mixed age (MA) ewes, 45% had diseased two-tooth (TT) ewes, and 5% had diseased hogget (H) ewes.

Salmonella Brandenburg appeared to occur in better performing flocks, which are often associated with intensive farming methods. At the farm level, factors such as increased total number of ewes, feeding of hay, and controlled winter grazing appeared to increase the risk of disease. Farming methods such as controlled winter grazing may result in higher stress levels and increase the shedding of *Salmonella* Brandenburg organisms. This may create a higher risk of exposure in sheep yards and on pasture, resulting in a higher risk of disease. Feeding crop and having hilly terrain decreased the risk of a farm having disease. A protective effect of hilly terrain could be due to less intensive farm management, with a subsequent reduction in stress associated disease risk. Within affected farms, disease appeared to be more severe with the removal of rams after July, feeding of hay, and the practices of strop grazing. Shearing after July, increasing the total number of pre-lamb yardings, and vaccinating for *Salmonella* appeared to be protective. Therefore reducing stress and vaccinating ewes appear to reduce the risk of a *Salmonella* Brandenburg outbreak.

DEDICATION

To Isabella Kerslake

Always wishing that I could have been closer.

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I arrived at the Epicentre as a bit of a lost soul, unsure in what I wanted to do in the year 2000, let alone what I wanted to do in life. It was at the Epicentre where that all changed.

I met and knew so many amazing and interesting people during my Masters that influenced and supported me in a number of fantastic different ways. I am grateful to everyone; in whatever little or huge parts that you played in the past couple of years of my life, I could not have grown or become half the person that I am today without all of you.

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