Surveillance and risk assessment for ovine Johne’s disease in Australia

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Statement of Sources

I hereby declare that the work presented in this thesis is, to the best of my knowledge and belief, original and my own work, except as acknowledged in the text, and the material has not been submitted, either in whole or in part, for a degree at this or any other university.

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Evan SG Sergeant

19 August, 2003
Abstract

Ovine Johne’s disease (OJD) was first diagnosed in Australia in 1980, and by January 1998, more than 200 infected flocks had been identified in New South Wales, Victoria and South Australia. In 1998, in response to the increasing number of infected flocks and industry concerns about the spread of the disease, a 6-year, AUD 40.1 million national program commenced, with the aim of controlling the disease while further research was undertaken to evaluate future control or eradication options. This research was undertaken as part of the national OJD program, to improve our understanding of the performance of existing and new diagnostic tests for the disease, to estimate disease prevalence and distribution and to develop a simple, objective system for assessing flock-risk of infection.

In the first study, the sensitivities and specificities of an absorbed enzyme-linked immunosorbent assay (ELISA) and an agar-gel immuno-diffusion (AGID) test for the detection of Johne’s disease in sheep were estimated using data from six known infected and 12 assumed uninfected sheep flocks. Logistic regression analysis was used to test for significant effects of lesion score and condition score, with flock included in the model as a random effect.

Estimated specificities were 95% (95% CI: 93.4, 95.6%) and 99% (98.4, 99.4%) for ELISA cut-point ratios of 2.4 and 3.6 respectively, and 100% (99.7, 100.0%) for the AGID. Estimated sensitivities were 41.5% (35.0, 48.3%), 21.9% (16.6, 27.9%) and 24.6% (19.1, 30.7%) for ELISA cut-point ratios of 2.4 and 3.6 and for AGID, respectively. ELISA and AGID sensitivities varied between flocks and between categories of lesion score and condition score. Sensitivity was highest in thin sheep and
in sheep with multibacillary lesions. The effects of lesion type and condition score on test sensitivity were both significant in the logistic regression analysis for the AGID and ELISA at both cut-points and the flock effect was significant for the AGID but not for the ELISA at either cut-point.

In the second study, the flock-level sensitivity of pooled faecal culture and serological testing using AGID were compared in 296 flocks in New South Wales during 1998. The overall flock-sensitivity of pooled faecal culture was 92% (82.4%, 97.4%) compared to 61% (50.5%, 70.9%) for serology (assuming that both tests were 100% specific). In low-prevalence flocks (estimated prevalence <2%), the flock-sensitivities of pooled faecal culture and serology were 82% (57%, 96%) and 33% (19%, 49%) respectively, compared to 96% (85%, 99.5%) and 85% (72%, 93%) respectively in higher-prevalence flocks (estimated prevalence ≥2%).

In a third study, the results of abattoir surveillance for OJD carried out during 2000 were analysed to estimate the prevalence of infected flocks in three regions of New South Wales and in other States. A Bayesian approach was used to adjust apparent prevalence estimates for the assumed flock-sensitivity and flock-specificity of abattoir surveillance, and to allow for uncertainty about the true values of these measures. The 95% probability limits for flock-prevalence at 31 December 2000 were 0.04% – 1.5%, 8% – 15% and 29% – 39% for low, moderate and high prevalence regions of New South Wales respectively. The other States generally had an upper 97.5% probability limit of about 1% or less. Based on this analysis, there were probably between 2,000 and 3,700 infected flocks in Australia at 31 December 2000, with more than 80% of these in a relatively small geographic area of central and southern New South Wales.
The final part of the current research was to develop a simple quantitative model for flock-risk of OJD, based on estimated flock-prevalence and within-flock prevalence for different classes of flocks, depending on their location and status. A method is also described for modifying this risk-score for individual flocks, according to the presence and level of individual flock risk factors such as the use of vaccination, testing history and the presence and number of infected neighbours. This flock-based approach to risk assessment could be supported by varying degrees of regulatory control over sheep movements, or could be adapted to a deregulated environment, with sheep producers taking responsibility for their own risk management, rather than the State or Territory regulatory authorities.
Acknowledgments

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### Acronyms and abbreviations

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<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AGID</td>
<td>Agar-gel immuno-diffusion test</td>
</tr>
<tr>
<td>CMI</td>
<td>Cell-mediated immunity</td>
</tr>
<tr>
<td>CT</td>
<td>Check tested — Pooled faecal culture of 2 pools of 50 sheep or serology on 250 sheep with negative results within the last 12 months</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immuno-sorbent assay</td>
</tr>
<tr>
<td>LP, MP, HP</td>
<td>Low, moderate and high prevalence</td>
</tr>
<tr>
<td>MN1, MN2,</td>
<td>Monitored Negative 1, 2 or 3 status, respectively, in the SheepMAP</td>
</tr>
<tr>
<td>MN3</td>
<td></td>
</tr>
<tr>
<td>OJD</td>
<td>Ovine Johne’s disease</td>
</tr>
<tr>
<td>NOJDP</td>
<td>National Ovine Johne’s Disease Control and Evaluation Program</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PFC</td>
<td>Pooled faecal culture</td>
</tr>
<tr>
<td>REA</td>
<td>Restriction endonuclease analysis</td>
</tr>
<tr>
<td>SDRs</td>
<td>National Johne's Disease Program Standard Definitions and Rules for Sheep</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>SheepMAP</td>
<td>The Australian Johne's Disease Market Assurance Program for Sheep</td>
</tr>
</tbody>
</table>
Introduction

Johne’s disease, or paratuberculosis, is a syndrome characterised by chronic, granulomatous enteritis affecting mainly ruminants, and is caused by *Mycobacterium avium* subsp. *paratuberculosis*. Johne and Frothingham first described the condition as an atypical form of tuberculosis in cattle in 1895 (Johne and Frothingham, 1895, cited by Chiodini et al., 1984) and Bang subsequently named the condition pseudo-tuberculosis, or Johne’s disease, in 1905 (Bang, 1906, cited by Chiodini et al., 1984). The first case of Johne’s disease in sheep was reported in 1911 (Stockman, 1911). Johne’s disease now has a virtually worldwide distribution in farmed ruminants and has also been diagnosed in deer and South American camelids, as well as many species of wild ruminants (Kennedy and Benedictus, 2001). *M. a. paratuberculosis* has also been isolated from a small number of humans suffering from Crohn’s disease, although there is still considerable debate about the significance of this finding in the aetiology of Crohn’s disease (Anonymous, 2000a; Chiodini and Rossiter, 1996).

Ovine Johne’s disease (OJD), due to infection with sheep strains of *M. a. paratuberculosis*, was first diagnosed in Australia in 1980 (Seaman et al., 1981). By January 1998, more than 200 infected flocks had been identified in New South Wales, and infection had also been confirmed in Victoria and South Australia, although the disease appeared to be geographically clustered (Sergeant, 2001). During 1998, in response to the increasing number of infected flocks and industry concerns about the spread of the disease, a 6-year, AUD 40.1 million national program for the control of Johne’s disease in sheep commenced. The program was funded jointly by Commonwealth and State governments and sheep industries, with the aim of controlling
spread of the disease while further research was undertaken to evaluate future control or eradication options (Allworth and Kennedy, 2000).

**Objective**

The objective of this research was to support the national program by:

1. Providing improved estimates of the sensitivity and specificity of screening tests used for surveillance and market assurance testing;
2. Providing estimates of the prevalence of infected flocks on a state or regional basis, to support zoning and to assist in future decision-making; and
3. Evaluating the risk of infection associated with various categories of flock, based on location and flock history, to support the development of trading options based on individual flock risk rather than geographic risk.

**Thesis structure**

The rest of this thesis is set out in four parts, as follows:

Part 1 provides a literature review and other background information. Chapter 1 provides a review of the epidemiology and pathogenesis of Johne’s disease in sheep (from Sergeant and Whittington, 2003, submitted), while Chapter 2 provides a brief history of OJD in Australia, updated from Sergeant (2001).

Part 2 provides an evaluation and comparison of the key flock-screening tests used for surveillance and market-assurance testing under the national program. The main serological test (the agar-gel immuno-diffusion test – AGID) is evaluated in Chapter 3, along with an alternative enzyme-linked immuno-assay (ELISA) (from Sergeant et al.,
Chapter 4 compares the performance of culture of pooled faecal samples and the AGID as flock-screening tests for OJD (from Sergeant et al., 2002).

Part 3 uses simulation methods to estimate the flock-prevalence of OJD in Australia on a regional and state basis, and the risk of infection associated with sheep from flocks categorised by location and other risk-factors. Chapter 5 provides estimates of the prevalence and distribution of infected flocks at 31 December 2000 (from Sergeant and Baldock, 2002), while updated estimates taking account of additional surveillance during 2001, are presented in an Appendix (Sergeant, 2003, in press). In Chapter 6 a simple, objective risk-assessment approach is used to develop a basis for risk-based categorisation of flocks, with potential to include a variety of important risk factors for the likelihood of infection in individual flocks (from Sergeant et al., 2003a).

Finally, Part 4 (Chapter 7) concludes the thesis with a discussion of the key findings and conclusions from the research, and the impact of the findings on the National Ovine Johne’s Disease Control and Evaluation Program and on the likely future direction of OJD control in Australia.
Part 1

Review of the epidemiology and occurrence of OJD in Australia
1. Epidemiology and pathogenesis of Johne’s disease in sheep

1.1 Introduction

In recent years there has been increasing interest in many countries in developing control programs for Johne’s disease in both cattle and sheep (Kennedy and Benedictus, 2001). In Australia, a national program, funded jointly by governments and industry, commenced in 1998 with objectives of limiting further spread of Johne’s disease in sheep, undertaking research into the epidemiology of the disease, and better defining its prevalence and distribution (Allworth and Kennedy, 2000).

This chapter reviews the pathogenesis and epidemiology of Johne’s disease in sheep, drawing on recent reviews and current research in Australia. Far more is known currently about Johne’s disease in cattle than in sheep, and for this reason some conclusions are extrapolated from research in cattle.

1.2 Biology of *M. paratuberculosis*

*M. paratuberculosis*, the causative organism of Johne’s disease, is a Gram-positive, acid-fast bacterium, closely related to *Mycobacterium avium*. It is defined as an obligate pathogen of animals and requires mycobactin as an exogenous source of iron for growth and replication in-vitro (Chiodini et al., 1984; Thorel et al., 1990). *M. paratuberculosis* can be differentiated from *M. avium* based on cultural characteristics and DNA analysis using the IS900 insertion sequence that has been considered specific to *M. paratuberculosis* (Collins et al., 1990; Vary et al., 1990; Whipple et al., 1990).

However, a small number of mycobacteria other than *M. paratuberculosis* that were
positive in assays to detect IS900 by PCR have recently been identified, so that a secondary test such as sequencing or restriction endonuclease analysis of PCR product should be undertaken to confirm the identity of PCR-positive isolates (Cousins et al., 1999; Englund et al., 2002).

### 1.3 Transmission of infection

Spread of paratuberculosis is primarily via the faecal-oral route, with clinically affected animals excreting large numbers of organisms and causing significant environmental contamination. Young animals are exposed to faecal contamination of the udder, fodder and the environment, providing ample opportunity for exposure. Dissemination of infection to tissues beyond the intestine does occur, including to the uterus, supramammary lymph nodes, udder and sexual organs. The organism may be excreted in milk and semen (Stehman, 1996; Sweeney, 1996; Eppleston and Whittington, 2001), and intra-uterine infection of the foetus has been confirmed in cattle (Sweeney, 1996). The level of foetal infection ranged from 8.6% of foetuses in asymptomatic cows to 20% – 40% in clinical cases (Sweeney, 1996). However, although some calves may be infected in utero or directly from infected milk, this does not appear to be a major source of spread (Chiodini et al., 1984; Sweeney, 1996). Although there is a report of vertical transmission in sheep (Tamarin and Landau, 1961), its importance is still unclear, but is probably relatively minor compared to horizontal transmission to lambs, during and soon after lambing via faecal contamination of the udder and environment.

#### 1.3.1 Susceptibility to infection

Age is the factor most commonly regarded as affecting susceptibility to infection. In cattle, susceptibility to infection appears to be highest in young animals and declines progressively with age. Cattle are generally assumed to be resistant to infection by
about one year of age, and infection of adult cattle may require much higher infective doses and result in longer incubation periods than is the case with neonatal infection (Whitlock and Buergelt, 1996). Similar age-related resistance is assumed to occur in sheep, although this has not been confirmed and natural infection of adults has been observed in Australia.

Variations in susceptibility between breeds of cattle have also been suggested, although this may relate to abundance of the breed and management factors affecting transmission and progression of infection rather than true differences in susceptibility (Chiodini et al., 1984). Breed-differences in susceptibility in sheep have not been documented. However, there have been anecdotal reports of variations in the level of disease between breeds in Australia, with British breeds and their crosses regarded as less prone to clinical disease than Merinos.

1.3.2 Infectious dose

The infective dose of *M. paratuberculosis* is not known, but appears to be fairly low (Chiodini et al., 1984). Infection may result from single or multiple oral exposures, and the infectious dose for sheep may be as low as $10^3$ – $10^7$ organisms (Brotherston et al., 1961; Reddacliff et al., 2001).

1.3.3 Excretion rate

Generally, animals in the early stages of infection are faecal-culture negative, although they may excrete *M. paratuberculosis* at rates below the limits of detection for current culture techniques (Whitlock and Buergelt, 1996). However, compared to the high levels of excretion from more advanced cases, early (latent) cases probably do not contribute significantly to the overall level of environmental contamination. As the
infection progresses, the level of faecal excretion of *M. paratuberculosis* also increases. Initially, excretion may be intermittent, becoming more constant as the disease progresses (Chaitaweesub et al., 1999; Whittington et al., 2000c). The average excretion rate for five sub-clinically infected sheep in one study was $1 \times 10^8$ organisms per gram of faeces, or about $8 \times 10^{10}$ organisms per sheep per day (Whittington et al., 2000c). Excretion rates for earlier cases (intermittent and light shedders) have not been quantified. Faecal shedding can commence as early as 9 months of age, but more commonly is not seen until after 12 to 18 months (Chaitaweesub et al., 1999; Eppleston et al., 2001).

### 1.3.4 Survival of *M. paratuberculosis* in environment

*M. paratuberculosis* is capable of surviving for extended periods in the environment, in faeces, soil or water. Survival has been reported for 8 – 11 months in bovine faeces, and for up to 17 months in tap water at a range of pH values (5 to 8.5) (Vishnevski et al., 1940, cited by Wray, 1975; Lovell et al., 1944; Larsen et al., 1956; Jorgensen, 1977). Under Australian conditions, sheep strains of *M. paratuberculosis* survived >12 months in faecal pellets in a shaded location, 4 – 9 weeks in unshaded environments during summer and for 48 weeks in the sediment of a water trough deliberately contaminated with infected faeces (Whittington, 2001). This potential for survival means that contamination rates build up while infected animals are present, and the environment remains potentially infective for susceptible livestock for many months after removing infected animals.

Exposure to direct sunlight is probably the main factor affecting the duration of survival of *M. paratuberculosis* in the environment, probably associated with temperature flux.
(Whittington, 2001). In these studies, moisture levels and lime application to increase soil pH had no apparent effect on survival.

### 1.4 Pathogenesis and progression of infection in sheep

Although the pathogenesis of Johne’s disease is still not well understood, it appears that it progresses through a number of stages, as described in Table 1.1. These hypothesised stages correspond to the changes that occur in histological lesions, and cellular and antibody-mediated immune response as the disease progresses. They correspond approximately with the stages of disease in cattle described by Whitlock and Buergelt (1996) and also with the range of histological lesions in sheep described by Perez et al. (1996) and Clarke (1997). Although described as distinct steps in the development of the disease, there is in fact no clear distinction between stages, but rather a gradual progression of the infection through successive stages.

Following exposure, infected animals undergo a variable, but generally long latent period (Chiodini et al., 1984; Stehman, 1996). It appears that some animals with latent infections may eliminate the infection without ever progressing, while others may remain in a latent or incubatory state throughout their productive life, without ever exhibiting clinical signs. In many flocks with established infection, it is likely that the majority of animals become infected, but that many of them subsequently eliminate the infection and are probably resistant to re-infection (Gilmour et al., 1978; Perez et al., 1996, Chiodini, 1996; Clarke, 1997).

After a variable period of time, a proportion of latent cases progress to become light shedders. The trigger to start this progression is unknown, but is possibly associated with waning of the cell-mediated immune (CMI) response. As lesions become more
Table 1.1. Stages of infection with Johne’s disease

<table>
<thead>
<tr>
<th>Classification</th>
<th>Duration a,b</th>
<th>Histological lesions c</th>
<th>Lesion type c</th>
<th>Stage b</th>
<th>% of herd/flock b</th>
<th>Gross lesions &amp; clinical signs c</th>
<th>Serological tests a</th>
<th>Tests for CMI d</th>
<th>Faecal shedding a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latent</td>
<td>Months – Years</td>
<td>Mild focal or multi-focal lesions, no visible afb’s</td>
<td>Type 1</td>
<td>Stage 1</td>
<td>60 – 70%</td>
<td>Nil</td>
<td>Ineffective, sensitivity close to zero (&lt;10%)</td>
<td>Not well characterised – potentially moderate-high</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Light shedders</td>
<td>Months</td>
<td>Spreading focal lesions, some with a few afb’s visible</td>
<td>Type 2</td>
<td>Stage 2</td>
<td>20 – 30%</td>
<td>Nil</td>
<td>Generally poor sensitivity (10-30%)</td>
<td>Sensitivity decreasing</td>
<td>Intermittent culture positive, low numbers of organisms</td>
</tr>
<tr>
<td>Heavy shedders</td>
<td>Weeks – Months</td>
<td>Progressive lesions, afb’s common</td>
<td>Type 3a</td>
<td>Stage 2</td>
<td></td>
<td>early gross lesions may be observed, no clinical signs</td>
<td>Moderate sensitivity (30-70%)</td>
<td>Poor sensitivity</td>
<td>Usually culture positive, increased concentration of organisms</td>
</tr>
<tr>
<td>Clinical</td>
<td>Weeks – Months</td>
<td>Advanced multibacillary (70 – 80% of cases) or paucibacillary (20 – 30% of cases) lesions</td>
<td>Types 3b, 3c</td>
<td>Stage 3</td>
<td>5 – 10%</td>
<td>Obvious gross lesions and clinical signs</td>
<td>High sensitivity in multibacillary cases (70 – 90%)</td>
<td>Poor sensitivity</td>
<td>Heavy, constant faecal shedding, very high concentration of organisms, except in paucibacillary cases</td>
</tr>
</tbody>
</table>

a Whittington and Sergeant, 2001; b Whitlock and Buergelt, 1996; c Perez et al., 1996; d Reliable estimates for sensitivity of CMI tests are not available for sheep (Collins, 1996; Hietala, 1992).
severe, animals start to shed the mycobacteria in their faeces, initially intermittently and eventually continuously, as the sheep progress through the various stages shown in Table 1.1. It may be possible for light or heavy shedders, or even clinical cases to recover and eliminate the infection, but this is probably a rare event (Hagan and Zeissig, 1935; Hagan, 1938; Gilmour et al., 1978). In sheep, clinical cases commonly occur in animals of 2-3 years of age, and sometimes as young as 14 months of age (Eppleston et al., 2002).

The factors affecting the probability and rate of progression through these stages are not fully understood. However, the (cumulative) dose of \( M. a. \) *paratuberculosis* received by an animal and the age at infection are thought to be important factors in affecting progression of infection (Whittington and Sergeant, 2001). Because CMI appears to have a key role in suppressing infection, any factors affecting the ability of an animal to maintain an effective CMI response are also likely to affect progression of disease (Chiodini, 1996).

Given the long incubation period and chronic nature of the disease, only a very small proportion of the infected sheep in an infected flock will show signs of clinical disease at any one time. The majority of infected sheep will have either latent or sub-clinical infection, as described for cattle (Whitlock and Buergelt, 1996). Many sheep may also have recovered from the infection and be immune, while some may be excreting \( M. a. \) *paratuberculosis* but still be seronegative.

### 1.4.1 Effect of soil type and pH on expression of Johne’s disease

The effect of soil type and pH on the occurrence of Johne’s disease is still unclear, despite a number of reports suggesting an association. This is further complicated
because the likely mechanism of action is also unclear. It has been hypothesised that high pH and low availability of iron in the soil may affect organism survival and growth in the environment (Johnson-Ifearulundu and Kaneene, 1997), and conversely that low pH may increase organism viability by increasing iron availability. However, this appears unlikely because pH appears to have only a slight effect on organism survival in water, and application of lime did not affect survival under experimental conditions (see section 1.3.4). Also, because *M. paratuberculosis* is defined as an obligate pathogen of animals, it is assumed not to undergo multiplication or growth in the environment. An alternative hypothesis is that high soil pH acts to reduce the level of available iron in the animal, limiting the ability of the organism to multiply and cause disease in infected animals (Richards, 1989b). As early as 1935, an association between low soil pH and clinical or serological evidence of Johne’s disease in cattle was reported in England, with subsequent reports from France, the Netherlands, United States of America, South Africa and Spain also supporting this hypothesis (Smythe, 1935; Jansen, 1948; Gasse, 1962, cited by Kopecky, 1977; Kopecky, 1977; Richards, 1989a; Michel and Bastianello, 1999; Reviriego et al., 2000). The application of lime to pastures has also been suggested as being protective against Johnes’s disease. Clinical Johnes’s disease is reported to have disappeared from the island of Jersey after the adoption of widespread liming of pastures in the early 1900’s (Spicer, 1936), and liming of pastures was associated with a reduction in the likelihood of being sero-positive for Johnes’s disease in US dairy herds (Johnson-Ifearulundu and Kaneene, 1998).

However, some of these reports are anecdotal in nature, and are not supported by detailed data to support the hypothesis (Smythe, 1935; Spicer, 1936; Gasse, 1962; Richards, 1989a). Other reports present more detailed analyses, but do not effectively
demonstrate that the observed differences between regions or herds were not due to other causes, particularly the underlying distribution of the infection due to historical or industry-related factors or to other measures implemented concurrently with liming. In Australia, the main OJD-endemic region is one of mostly acid soils, making it very difficult to evaluate the influence of soil type.

1.5 Clinical signs and pathology

1.5.1 Clinical signs
The most consistent clinical sign associated with Johne’s disease is progressive wasting, emaciation and eventual death of affected animals. Diarrhoea, although common in cattle, is only occasionally seen in sheep and goats. In advanced cases a protein-losing enteropathy develops, resulting in hypoproteinaemia and sub-cutaneous oedema, particularly of the sub-mandibular area (bottle jaw). Appetite tends to remain good until late in the course of the disease. The clinical course of the disease may last from a few weeks to several months, from first clinical signs until death. None of these signs are specific for Johne’s disease, and internal parasitism and mineral deficiencies may cause similar presentations. Onset of clinical signs is often triggered by stress factors such as parturition, malnutrition or intestinal parasitism.

1.5.2 Gross pathology
Common findings at post mortem include severe emaciation of the carcase, sub-mandibular oedema, serous atrophy of fat deposits, and serous fluid accumulation in body cavities. The main lesions of Johne’s disease are usually limited to the intestinal tract and associated lymph nodes. The intestinal wall is usually thickened, often with corrugation of the mucosal surface. Intestinal lesions may be localised or diffuse, but are most commonly associated with the terminal ileum and ileo-caecal valve area.
Associated lymph nodes are often enlarged and oedematous, and lymphatic vessels draining affected intestine may be corded and thickened.

1.5.3 Histopathology

Two main types of lesions have been described in clinical cases of Johne’s disease in sheep. The most common lesion (about 70 – 80% of clinical cases) is a diffuse accumulation of epithelioid cells, usually accompanied by large numbers of acid-fast organisms visible in the lesions. This lesion type is generally referred to as a lepromatous, or multibacillary lesion. The less common lesion type is one of discrete lymphoid granulomas containing few, if any, acid-fast organisms. These lesions are generally referred to as tuberculoid, or paucibacillary type (Carrigan and Seaman, 1990; Clarke and Little, 1996).

A wider range of lesions has been described in sheep culled from four known infected flocks (Perez et al., 1996). Type 1 lesions, representing about 50% of infected sheep, were small granulomata associated exclusively with the ileo-caecal valve Peyer’s patch. No gross lesions or acid-fast organisms were observed in sheep with Type 1 lesions. Type 2 lesions were seen in about 10% of cases. These lesions were similar to Type 1 lesions, but more extensive. Again, there were no gross lesions seen in sheep with Type 2 lesions, although a few acid-fast organisms were seen in two of eight sheep with these lesions. There was no evidence of clinical Johne’s disease in sheep with either Type 1 or Type 2 lesions. Type 3 lesions were further subdivided into three sub-categories. Type 3a lesions were seen in about 9% of infected sheep. These lesions were similar to Type 2, but more severe, with acid-fast organisms more common and mild gross lesions apparent. Type 3b lesions were present in about 28% of infected sheep. These lesions were typically characterised by diffuse epithelioid infiltration with numerous acid-fast
organisms present, corresponding to the lepromatous, multibacillary type lesions described previously as occurring in the majority of clinical cases. All animals with these lesions had severe gross lesions, and were showing clinical signs typical of Johne’s disease. Type 3c lesions were diffuse lymphoid lesions, with few acid-fast organisms present, occurring in about 5% of cases. Animals with Type 3c lesions also had severe gross lesions, similar to those with Type 3b. Type 3c lesions were typical of the tuberculoid, paucibacillary lesions reported previously as occurring in a minority of clinical cases.

1.6 Losses due to disease

Johne’s disease causes progressive wasting and eventual death in clinically affected animals, and additional production losses in sub-clinical cases. Although the clinical effects of the disease are generally obvious, direct disease-related losses from Johne’s disease are difficult to estimate because of the prolonged sub-clinical period, and the difficulty in accurately attributing the cause of production losses and death in many cases. In sheep, the direct losses are mainly associated with increased mortality rates due to the occurrence of clinical cases, decreased wool and milk production and reduced fertility and lamb survival in clinical and pre-clinical cases. Additional indirect costs may occur due to having fewer excess sheep for sale and, in Australia, lost trading opportunities because of quarantine of known or suspected infected flocks.

1.6.1 Mortality rates

Estimates of mortality rates due to ovine Johne’s disease vary considerably, and are mainly anecdotal, based on farmer reports. Estimation is further complicated by the need to distinguish mortalities due to Johne’s disease from those that die with Johne’s disease and those that are unrelated to Johne’s disease (McGregor et al., 2001). Under
Australasian conditions, mortalities generally increase over time, and commonly reach 5% – 15% per annum and occasionally higher (Eppleston and Simpson, 1999; Eppleston et al., 2000). Detailed investigations in one high-prevalence New South Wales’ flock found an estimated annual mortality rate due to Johne’s disease of 14.6%, in sheep over 12 months of age (McGregor et al., 2001). Reported annual mortality rates due to Johne’s disease in other countries range from <1.6% in New Zealand (West, 1997) to an average of 8-9% and up to 40% in some individual flocks in Iceland (Fridriksdottir et al., 2000).

1.6.2 Production losses

In cattle, sub-clinical Johne’s disease is known to cause decreased milk production in the lactation(s) prior to onset of clinical signs, as well as reduced fertility and increased incidence of mastitis. Reductions in milk production of 6% – 16% have been recorded in cows prior to onset of clinical signs (Kennedy and Benedictus, 2001).

In sheep in Spain, ELISA positive ewes produced about 10% less milk than ELISA negative cohorts (Aduriz et al., 1994). In Australia, ELISA positive sheep gained weight at about 7 grams/day less than ELISA negative sheep (Chaitaweesub et al., 1999). In the same study there was no difference in wool production between ELISA positive and negative groups. A reduction in fertility and decreased wool production and wool quality in sub-clinically infected sheep are also likely but have not been confirmed.

1.7 Infection in other species

Detailed DNA analysis has shown that there are numerous strains of *M. paratuberculosis*, which can be broadly categorised into two groups, identified as C (cattle) and S (sheep) (Collins et al., 1990). C types have been isolated from most
ruminant species, while S types have been isolated mainly from sheep, with infection of cattle, deer and goats being less common (Whittington et al., 2000b). Thus, there appears to be a host-preference for the different strain types, with this preference not being absolute.

Natural infection of rabbits, foxes, stoats and other wildlife with \textit{M. a paratuberculosis} has also been recorded (Greig et al., 1997; Beard et al., 1999; Beard et al., 2001; de Lisle et al., 2002). A number of mono-gastric species have also been infected experimentally, although infection did not usually result in occurrence of clinical disease or typical lesions of paratuberculosis (Chiodini et al., 1984; Sharp, 1997). Although natural infection may occur in a wide range of free living species, the significance of such infection in the epidemiology of the disease in livestock is still unclear.

In Australia, there has been no evidence of infection in rabbits, despite testing of >600 rabbits from 13 farms in New South Wales and Victoria — two farms infected with bovine Johne’s disease and 11 with ovine Johne’s (Abbott, 2000; Kluver et al., 2000). Testing of 300 kangaroos from 10 Johne’s disease-infected properties in New South Wales found one animal that was faecal-culture positive but histologically negative, with the remaining animals negative on either faecal culture or histology (Abbott, 2000). The one culture-positive animal had only low numbers of organisms in its faeces, and was thought to be passively excreting organisms acquired from contaminated pasture, rather than actively infected. Based on these results, the estimated prevalence of infection in both rabbits and kangaroos was 0% with upper 95% confidence limits of
0.6% and 1.2% respectively, assuming that the culture positive kangaroo was not infected.

1.8 Spread between flocks

In most countries, sheep are traded frequently and this is the most common means of spread of the infection. In Australia the current regional pattern of infection matches common sheep trading patterns. In 2001, an analysis of 2,230 movements of sheep off known infected properties in New South Wales found that only 6% of all movements were to properties in the low-prevalence region (<0.5% of flocks known to be infected) of New South Wales, compared to 45% and 49% to the moderate (0.5 – 5% of flocks known to be infected) and high-prevalence (>5% of flocks known to be infected) regions, respectively (see Figure 1.1) (Baldock and Sergeant, 2001). The main mechanism of spread of Johne’s disease between farms, particularly for the introduction of infection into previously uninfected areas, is through the movement of infected sheep. However, direct farm to farm environmental spread, such as by water, or spread by fomites is possible but has never been confirmed (Sweeney, 1996). Experience in Australia has shown that local spread of infection between neighbouring properties is very common, and that the risk of a flock being infected increases with the number of infected neighbouring flocks (Baldock and Sergeant, 2001). Although the mechanism of spread is unknown in most cases, straying sheep, common use of land or facilities, or movement of infected faeces in run-off water or wind are the most likely explanations.

Infected wildlife provide another possible means of local spread, although this is probably a minor factor. Macropods may contribute indirectly to spread through damage to boundary fences, allowing continuing straying of sheep. Other unlikely
mechanisms for local spread include spread of contamination by blowflies (Fischer et al., 2001) or nematode larvae (Lloyd et al., 2001; Whittington et al., 2001).

Figure 1.1. Distribution of movements of sheep off infected farms according to the district of origin and destination. Triangles indicate movements, with the base of the triangle in the district of origin and the point in the district of destination. The width of the base is proportional to the number of movements between respective districts.

1.9 Diagnosis

Issues related to diagnosis were reviewed recently (Whittington and Sergeant, 2001). Due to the poor sensitivity of the tests in early cases, and the production systems involved, diagnosis of paratuberculosis in sheep is usually made on a flock basis, rather than in individual animals. Generally, diagnosis relies either on investigation of suspect clinical cases, usually by post-mortem examination and histopathology, or the testing of a large sample of animals from the flock using a screening test such as serology or pooled faecal culture (Anonymous, 2001b). However, the latter form of diagnosis,
which is required in regional disease control programs, poses a difficult challenge, because of the long incubation period and the generally low-moderate sensitivity of the available screening tests, particularly in early cases. During the early stages of infection, when the infection is dormant or just starting to progress, CMI dominates the animal’s response to infection and serological tests will be negative (Clarke, 1997). Some early cases may be excreting low levels of *M. avian paratuberculosis* in their faeces, often intermittently, and so may also be difficult to detect by faecal culture. As the infection progresses, excretion of *M. avian paratuberculosis* increases, and animals are likely to be faecal culture positive for many months before becoming seropositive or showing signs of disease (Chaitaweesub et al., 1999; Whittington and Sergeant, 2001).

All tests have imperfect sensitivity and/or specificity. Tests for CMI, such as the gamma-interferon test, provide the best hope for early detection of infection in live animals, before they have had an opportunity to spread the infection, but these tests have not been validated. Pooled faecal culture also appears to be a highly sensitive test and is capable of detecting flock-infection very early at low cost. However, because this is a culture-based test that requires about 3 months incubation, infected animals will be excreting significant numbers of organisms before confirmation of infection, providing opportunities for further spread of the disease. Serological tests such as the agar-gel immuno-diffusion test have very poor sensitivity until quite late in the course of disease, and therefore are better suited to identification of animals for post-mortem examination and rapid confirmation of infection in flocks with well-established infection.

### 1.10 Control of infection on-farm

Effective control of infection on-farm depends on reducing direct or indirect exposure of susceptible sheep (particularly lambs and weaners) to infected faeces, and slowing
the progression of infection in animals that do become infected. In dairy cattle this is possible through calf-management programs to isolate susceptible calves from adult faeces (Collins, 1994; Kennedy and Benedictus, 2001). Under intensive husbandry conditions, such as shed-rearing or winter housing, considerations for control will be similar to those applied in cattle. However, such an approach is not feasible for sheep under extensive grazing conditions, and alternative methods must be used. To date, there has been little research in this area, so there are few well-tested recommendations that are known to be effective.

On-farm control of Johne’s disease in sheep in Australia relies mainly on vaccination, in combination with management strategies such as rotation with other species or crops to interrupt transmission and reduce exposure of susceptible sheep. Other strategies, such as selective culling of known or suspected infected animals and their progeny/cohorts, segregation of the flock into high- and low-risk groups, purchase of replacement sheep from a low-risk flock rather than keeping home-bred replacements and liming of soils may help control the infection, but have not been fully evaluated.

1.10.1 Vaccination

Vaccination currently provides the best prospects for effective on-farm control of ovine Johne’s disease. Vaccination of lambs appears to reduce the levels of both faecal shedding of *M. paratuberculosis* and of clinical disease in infected flocks (Sigurdsson, 1960; Chiodini et al., 1984; Cranwell, 1993; Juste et al., 1994; Juste, 1997). Vaccination has also provided very effective long-term control but not eradication of Johne’s disease in sheep and goats in Iceland (Fridriksdottir et al., 2000). Preliminary results from vaccination trials in Australia support the effectiveness of vaccination as a control measure, with both Johne’s disease mortalities and faecal shedding of *M. a*
delayed and reduced in vaccinated sheep, compared to unvaccinated cohorts (Eppleston et al., 2002). Vaccination of adult sheep may also be effective in reducing subsequent losses due to disease (Crowther et al., 1976; Corpa et al., 2000). However, adult vaccination may not always be effective (McGregor et al., 2002) and it is not recommended in Australia.

There are some drawbacks to use of the vaccine, including the occurrence of persistent injection-site lesions that can result in downgrading of carcases, severe injection-site reactions in humans following accidental injection, interference with immunological tests for the presence of humoral or cellular immunity to *M. paratuberculosis*, *M. bovis* and *M. tuberculosis* and potential human health risks associated with the use of live attenuated vaccine formulations (MacDiarmid, 1987; Juste et al., 1994; Gwozdz et al., 2000; Eppleston et al., 2001). Although vaccination is not 100% effective in preventing infection, it does reduce excretion rates, and over time will significantly reduce overall levels of contamination on infected properties. The result will be a reduction in disease severity and an example is the elimination of clinical disease from sheep in Iceland following a long-term vaccination program (Sigurðarson and Gunnarsson, 1983). Simulation modelling has also suggested that eradication is a possible outcome from long-term vaccination in individual flocks (Juste and Casal, 1993; Sergeant, 2002a). However, vaccination will mask the obvious signs of the infection and make it more difficult to detect using immunological tests and faecal culture.

### 1.11 Conclusion

Additional research is still required to fully understand the epidemiology and pathogenesis of Johne’s disease in sheep. An improved understanding of factors affecting the establishment and progression of infection are essential for the
development of effective strategies for the control and eventual eradication of this infection on infected farms. Similarly improved diagnostic tests, capable of detecting infection before faecal excretion occurs, would substantially improve the opportunities for control of this disease. However, even with current knowledge and tools, it is possible to manage the disease and reduce its impact in infected flocks.
2. History of ovine Johne’s disease in Australia

2.1 Introduction and spread of OJD

This chapter describes the history of OJD in Australia since the first case was identified in 1980 (Seaman et al., 1981), and the response of government and industry to the spread of this disease, up until the end of 2002.

OJD was first confirmed in sheep in Australia in 1980, on a property in the central tablelands region of New South Wales (Seaman et al., 1981). Since 1980, the disease has spread slowly to other areas of New South Wales and, by early 1999, it had also been identified in Victoria, on Flinders Island in Tasmania, on Kangaroo Island in South Australia and a single introduced case had been identified in mainland South Australia (Allworth and Kennedy, 2000). By 31 December 2002, 1,765 infected flocks had been identified in Australia, with only Queensland and the Northern Territory not having recorded any cases (Allworth, 2003).

The origin of the sheep infection in Australia has never been determined, although the New South Wales cases were presumed to originate from imported sheep, probably introduced during the 1960’s or ‘70’s from New Zealand (Denholm et al., 1997). However, importation of sheep into Australia from any country was prohibited from 1958 until 1974, when the ban was relaxed to allow the importation of sheep from New Zealand (O’Neill, 1973; Miller, 1974). Between 1975 and 1979 many carpet-wool sheep were imported from New Zealand into Australia (Anonymous, 1979), and particularly into the central tablelands area of New South Wales within a few kilometres of the first known infected farm (A Kajons, personal communication).
At the time of detection of the first case in New South Wales, extensive tracing was undertaken to try and identify the source, without success. The infection appeared to have been present on the property for several years prior to diagnosis, and no link to imported sheep could be established. A subsequent survey of flocks in the district found an additional five infected flocks by the end of 1982 (Seaman and Thompson, 1984). Intensive investigation of all these flocks failed to find any links to imported sheep, or a common source, and the disease appeared to have been present in the district for at least 3-4 years prior to being detected. Reports at the time from official veterinarians suggested that cases of emaciation and scouring in individual adult sheep, with gross thickening of the ileum and sometimes rectum, had been observed in the district during the 1960’s and 1970’s, although the cause of these cases had not been established (H Scott-Orr, personal communication). Thus, it appears that OJD may have been introduced as late as 1975, in the early importations of carpet wool sheep, or more likely, may have been present and slowly spreading in the district since the 1950’s.

Studies on some of the early cases identified in New South Wales confirmed that the disease seen in sheep in Australia is epidemiologically quite different from that in cattle. The disease in sheep has a very different geographic distribution from that in cattle and *M a paratuberculosis* has been difficult to culture from affected sheep, as has been observed with sheep strains overseas (Carrigan and Seaman, 1990; Seaman and Thompson, 1984). Since the development of reliable techniques for culture of sheep strains and for strain typing of isolates, DNA studies have shown that all of 142 isolates from sheep were sheep strains of *M a paratuberculosis* (Whittington et al., 2000b),
although a small number of cases of infection of sheep with cattle strains and *vice versa*
have now been confirmed.

Between 1980 and 1990, new infected flocks were identified at a rate of about 1-2 per
year in New South Wales (see Figure 2.1). All of the infected flocks identified in New
South Wales prior to 1992 were in the same central tablelands area, within 80 km of the
first case. In 1992, the first cases outside this area were confirmed in the Goulburn,
Young and Albury areas of southern and southwest New South Wales. Since then there
have been increasing numbers of infected flocks detected outside the central and
southern tablelands area, although this still remains the main focus of infection. By the
end of 1996 a total of 158 infected flocks had been identified in New South Wales, with
most of these in adjoining districts to the original case (Denholm et al., 1997). Increased
surveillance since 1996 has resulted in a rapid increase in the number of infected flocks
identified, so that by the end of 2002 a total of 1,379 infected flocks had been identified
(Allworth, 2003). This represents about 4.3% of the commercial sheep flocks in New
South Wales (Figure 2.1).

In 1989, the first case was identified outside New South Wales, on a property in the
west Gippsland region of Victoria. This was a single case in a ewe introduced from
Flinders Island (Millar, 1998). Extensive testing in this and other flocks at that time
revealed no further evidence of infection. Additional infected flocks were identified in
the east Gippsland area in December 1995 and in other areas of Victoria and on Flinders
Island, Tasmania during 1996. During 1997, the first infected flock in South Australia
was identified following tracing from a known infected flock, with a focus of infected
flocks identified on Kangaroo Island in South Australia during 1998. The first cases in the Australian Capital Territory were also confirmed during 1998, and an OJD-infected goat was identified in Western Australia during 2000 (J Edwards, personal communication). The origins of infection in Victoria and on Kangaroo and Flinders Islands are unknown, although the Flinders Island and at least some of the Victorian cases were probably due to separate importations, rather than spread from New South Wales (H Millar, R Andrewartha, personal communication).

Spread of OJD in Australia has been mainly due to the movement of infected sheep or by local spread between neighbouring flocks. There is no documented evidence of spread other than through contact with infected sheep, or grazing of land previously grazed by infected sheep, although indirect spread through movement of infected faeces between neighbouring properties cannot be ruled out. In New South Wales, very few foci of infection have been identified outside the central and southern tablelands areas

Figure 2.1. Annual detections of OJD in New South Wales to December 2000
that cannot be directly traced to the introduction of sheep from those areas (Links et al., 1999).

As a result of the apparent restricted distribution of the disease within States, there was increasing pressure from industry during the mid-1990’s to develop state or national programs to control or eradicate the disease while there was still an opportunity to limit its spread throughout Australia.

2.2 Initial response

Prior to 1995, little official action was taken to control or manage OJD, although some affected owners initiated voluntary OJD control or eradication programs on their farms. Identification of infected flocks relied entirely on the investigation of clinical cases by private or official veterinarians at the request of the owner. Infected farms were not routinely quarantined, and there were no coordinated surveillance or control programs for the disease.

In 1995, in response to the increasing number of cases of OJD, the New South Wales State government and sheep industry formed the New South Wales Johne’s Disease Sheep Industry Steering Committee. In 1996, the Steering Committee developed and implemented a strategic plan for the control and eradication of OJD (Anonymous, 1996). The plan proposed a two-stage approach to the control and eradication of OJD. The first stage was a control and surveillance program for 1 year, to limit further spread of the disease and determine its distribution. Known infected and suspect flocks were subject to movement restrictions to limit further spread, and movements of sheep onto and off known infected properties were traced and investigated. This stage also included proposals for development of a market assurance program and zoning within New
South Wales, as well as advisory and research programs. The second stage of the plan, eradication of the disease, was to proceed only if this was found to be feasible after completion of Stage 1.

Because the sensitivity of serological tests for OJD may be as low as 24% in some flocks (Garcia Marin et al., 1991; Huchzermeier and Bastianello, 1991; Marshall et al., 1996), random surveys to estimate prevalence and determine the distribution of the disease would have required large sample sizes and were not considered feasible or economic. Therefore, surveillance during this period relied mainly on investigation of properties identified by the tracing of sheep movements from known infected flocks and the investigation of suspect clinical cases. Progress under the Plan was also hindered by the lack of specific funding to support surveillance and control activities.

At the same time as the Strategic Plan was being implemented in New South Wales, Victoria was also embarking on an OJD Control Program (Millar, 1998). The Victorian OJD Control Program was agreed by the Victorian government and sheep industry in December 1996 and commenced in January 1997. The long-term objective of the Program was to minimise the impact of OJD on the Victorian sheep industry by progressive eradication of OJD on known infected sheep properties and by identifying flocks with a low risk of OJD as a source of replacement sheep for the industry in Victoria.

Financial assistance was provided to affected producers undertaking an approved eradication program, funded initially by a $1 million grant from the State government, and subsequently by an industry levy. Assistance was also provided to ensure that only
uninfected sheep were used to restock previously infected properties. By early 1998, 62 of 66 affected producers had developed an approved eradication plan and had commenced destocking their properties (Millar, 1998).

In all States, flocks known or suspected to be infected were subject to movement restrictions to limit the further spread of infection. Tracing of movements of potentially infected sheep onto and off infected properties was undertaken. Any properties identified by such tracing were treated as high-risk, and subjected to investigation to establish their true classification.

### 2.3 A national program

From mid-1997, discussions on a proposed national eradication program for OJD commenced between State and Commonwealth governments and industry organisations. Agreement on a national program could not be reached and in late 1997 the Commonwealth government commissioned an independent ‘assessment of the merit of a national OJD control and eradication program…..’. This report (The Hussey-Morris Report) recognised that, based on current knowledge of the disease, there was some uncertainty as to whether it could be eradicated (Hussey and Morris, 1998). Among other things, it recommended ‘a nationally coordinated program of combative and research action be pursued…’, including:

- a research program to evaluate on-farm eradication options;
- enhanced surveillance to identify infected flocks and better determine the distribution of OJD in Australia;
- programs to support claims of low-risk for OJD on a farm and regional basis;
- implementation of zoning and movement restrictions between zones of different
classification and

- resolution of deficits in our knowledge of the disease.

Following the recommendations of the Hussey-Morris report negotiations for a national program re-commenced in 1998. A nationally funded interim surveillance program for OJD was undertaken from 1 April 1998 until 31 August 1998, to provide additional data on the occurrence of disease, pending the implementation of a longer term program. Also during this period agreement was reached to proceed with a national program and a Business Plan for the program was developed.

In early 1999, the deeds of agreement to fund and implement a 6-year, $40 million National Ovine Johne’s Disease Control and Eradication Program (NOJDP), based on the recommendations of the Hussey-Morris Report, were signed. This program is jointly funded by the sheep industries (national and state) and Commonwealth and State governments and is managed by Animal Health Australia through a national program management committee. The program is coordinated by the National Johne's Disease Coordinator and implemented mainly by the various State governments. The broad aims of the program are to control the spread of OJD while further research is undertaken to determine the feasibility and cost-effectiveness of eradication, including determining the distribution and prevalence of the disease (Anonymous, 1998b).

2.4 Progress to 2002

2.4.1 Market Assurance Program

The SheepMAP is a voluntary program ‘to identify, protect and promote flocks that are at low risk of being infected with JD’ (Anonymous, 2000b). Veterinary Committee (the
Chief Veterinary Officers of the Commonwealth, State and Territory Governments) and Animal Health Australia endorsed guidelines for the SheepMAP in May 1997 (Anonymous, 1997), and updated guidelines in January 2000 and November 2002 (Anonymous, 2000b; Anonymous, 2002a). The SheepMAP provides for classification of flocks as Monitored Negative 1 (MN1), Monitored Negative 2 (MN2) or Monitored Negative 3 (MN3), in order of increasing confidence that the flock is free of the disease. To be classified as MN1 a flock must undertake testing of a statistically valid random sample of adult sheep using an approved screening test (Sample Test), with follow-up of any positive results. The level of testing required by the rules is sufficient to provide 98% confidence of detecting a prevalence of infection in the flock of 2% or greater, and currently approved tests are the AGID, ELISA and pooled faecal culture. Producers in the SheepMAP must also comply with rules designed to minimise the risk of introducing OJD into the flock and undergo an annual veterinary audit to maintain their classification (Anonymous, 1997; Anonymous, 2000b). A Sample Test of the flock is also required every second year to progress between categories. SheepMAP flocks may also elect to vaccinate lambs, to provide protection against infection and additional assurance of their low-risk.

By December 2002 a total of 748 flocks were classed as MN1 or better in the SheepMAP, representing about 0.9% of flocks Australia-wide (Table 2.1) (Allworth, 2003). Although the program is intended to provide a high level of assurance that assessed flocks are unlikely to be infected, some market assured flocks have subsequently been confirmed as infected. By December 2002, OJD had been confirmed in 37 SheepMAP flocks, 24 in New South Wales seven in Victoria, five in South Australia and one in Tasmania. Thirty five of these breakdowns were MN1 at the time.
they were detected, while one was MN2 and one MN3 (D Kennedy, personal communication). An additional two MN1 flocks had been identified as infected with the cattle strain of *M. a. paratuberculosis*.

Table 2.1. **Number of Infected, suspect and market assured flocks by zone at 31 December 2002 (Allworth, 2003).**

<table>
<thead>
<tr>
<th>Zone</th>
<th>Cumulative Infected</th>
<th>Current Infected</th>
<th>Suspect or Under Surveillance</th>
<th>MN1</th>
<th>MN2</th>
<th>MN3</th>
<th>Estimated number of flocks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual</td>
<td>880</td>
<td>782</td>
<td>2,468</td>
<td>12</td>
<td>54</td>
<td>66</td>
<td>4,738</td>
</tr>
<tr>
<td>Control</td>
<td>540</td>
<td>412</td>
<td>1,498</td>
<td>16</td>
<td>22</td>
<td>90</td>
<td>9,483</td>
</tr>
<tr>
<td>Protected</td>
<td>343</td>
<td>98</td>
<td>826</td>
<td>226</td>
<td>155</td>
<td>107</td>
<td>61,723</td>
</tr>
<tr>
<td>Free</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8,727</td>
</tr>
<tr>
<td>Total</td>
<td>1,765</td>
<td>1,292</td>
<td>4,800</td>
<td>254</td>
<td>231</td>
<td>263</td>
<td>84,671</td>
</tr>
</tbody>
</table>

2.4.2 **Standard Definitions and Rules**

Veterinary Committee also developed national standard definitions and rules (SDRs) for OJD, which were endorsed in early 1998 (Anonymous, 1998a). These SDRs have since been updated several times, with the fourth edition endorsed in May 2002 (Anonymous, 2002b). The SDRs provide guidelines and minimum standards for the management of OJD in Australia, and provide the basis for individual State or Territory control programs. They also underpin the development of zoning and movement controls within Australia and provide for classification of flocks according to their level of risk of being infected with OJD. In addition to the categories supported under the SheepMAP, the SDRs provide for classification of flocks into the following categories:

- **Infected**, where OJD has been confirmed in sheep on the property;
- **Suspect**, where there is epidemiological evidence to suggest that the flock may have been exposed to infection but it has not yet been confirmed;
- **Under Surveillance**, where there is evidence to suspect the disease, but after investigation the risk is determined to be low; and
- **Nil Assurance** (previously called Not Assessed), where a flock has no history of
OJD and has not been assigned any other classification.

2.4.3 Zoning

In July 1999, zoning for OJD was implemented across Australia, in accordance with the SDRs. Zoning was coordinated between States to ensure as little disruption as possible to the sheep industries of southeastern Australia. Initial zone declarations included:

- Residual Zones (OJD is endemic but some level of control is in place) in the central and southern tablelands of New South Wales and Flinders Island in Tasmania;
- Control Zones (OJD may be present at a manageable level and is actively controlled) for Victoria, South Australia, Queensland, Northern Territory and the balance of New South Wales and Tasmania;
- Protected Zones (OJD occurs only sporadically, and eradication measures are enforced). Western Australia was initially declared a Protected Zone and progressed to Free Zone classification in late 1999.
- Free Zones (there are no known or suspected infected flocks, and the absence of OJD has been demonstrated to the satisfaction of Veterinary Committee). Any infected flocks in Western Australia are destocked so that the State can continue as a Free Zone.

There is also provision for declaration of Infected Zones, where OJD is endemic and only minimal regulatory controls are enforced, although no OJD Infected Zones have been declared. Some Residual Zones were re-classified as Control Zones in early 2000, and Queensland, South Australia (except Kangaroo Island), Tasmania (except Flinders Island), Victoria and parts of New South Wales were re-classified as Protected Zones from 1 October 2002. The residual zone in New South Wales was also expanded.
slightly at this time. The Northern Territory does not have any commercial sheep flocks, but to facilitate the few sheep introductions that do occur from South Australia and Queensland is now regarded as a Protected Zone. Figure 2.2 shows the existing zones for OJD in Australia at 1 October 2002.

![Australian OJD Zoning Boundaries for implementation 1st October 2002.](image)

**Figure 2.2.** OJD zones across Australia as at 1 October 2002

### 2.4.4 Surveillance

Between 1996 and 1999, surveillance for OJD relied primarily on the testing of ‘at risk’ flocks identified by tracing the movement of sheep onto and off infected properties, or as neighbours of infected properties. As a result, surveillance concentrated on properties in known infected areas, or that had purchased sheep from these areas. From mid-1997,
market assurance testing provided additional surveillance data, including some data for areas where little testing had been done previously.

The introduction of abattoir surveillance in all States from late 1999 substantially increased the amount of surveillance in Control and Free Zones, providing much greater assurance of their status. Abattoir surveillance also provides an effective way of detecting flocks with established infection in these areas that is independent of tracing from known infected flocks, addressing concerns of possible independent foci of infection in these areas (Allworth, 2000). Abattoir surveillance for OJD is based on visual inspection of the intestinal tract of sheep after slaughter, with histopathological examination of tissue samples from any gross lesions suggestive of OJD. This technique shows considerable promise as a tool for non-targeted surveillance of sheep flocks, particularly in the endemic area. Abattoir inspection appears capable of easily detecting flocks with an established high prevalence of infection, but may be less sensitive when applied to low prevalence or recently infected flocks. Use of abattoir surveillance to support zone classification therefore depends on inspection of large numbers of sheep over sufficient time to provide confidence that the disease is absent.

By the end of 2002, more than 61,000 lines of sheep (comprising about 17.8 million sheep) had been examined by abattoir surveillance (Allworth, 2003), including lines from all sheep producing regions of Australia. For the quarter ending in December 2002, about 41% of lines from Residual Zones were found to be infected compared to 20% of lines from Control Zones and 2% of lines from Protected Zones.
Also during 1999, the use of pooled faecal culture was approved by Veterinary Committee as an alternative screening test to serology. A single faecal pellet is collected from each sheep sampled and pooled in lots of up to 50 pellets during collection. Pooled samples are then homogenised and cultured using the BACTEC system with \( M. \) paratuberculosis confirmed by PCR testing using the IS900 sequence or by sub-culture on solid medium (Whittington et al., 2000a). This test has a number of advantages over serology, including being cheaper and also more sensitive, particularly early in the course of infection (Whittington et al., 2000a). Because pooled faecal culture is based on the physical identification of \( M. \) paratuberculosis, this test is technically 100% specific, and a positive result should confirm that the flock of origin is infected.

However, pooled faecal culture has the disadvantage of taking up to 5 months to produce a final result in some cases, particularly where there is growth in the BACTEC medium and sub-culture on solid medium is required to confirm the result. The higher apparent sensitivity of pooled faecal culture makes it a very useful and economic tool for market assurance testing, for early clearance of suspect flocks, and for surveillance in low prevalence areas (Whittington et al., 2000a). Because pooled faecal culture is more sensitive at the individual animal level, the sampling rate for SheepMAP testing using pooled faecal culture (350 sheep) is lower than that for serology (875 sheep), but still provides the same level of confidence of detecting infection (Anonymous, 2002a).

As a result of the increased level of surveillance, the total number of infected flocks identified has increased from 197 in 1996 to 1,765 at the end of 2003 (Table 2.2) (Millar, 1998; Allworth and Kennedy, 1998; Allworth, 2000; Allworth and Kennedy, 2000; Allworth, 2001; Allworth, 2002; Allworth, 2003). By December 2002, about 2% of flocks had been confirmed as infected across Australia, 73% of which were still
classified as infected. A further 5.6% of flocks were classified as either suspect or under surveillance because of association through tracing or as a neighbour to an infected flock (Table 2.1) (Allworth, 2003). Overall, about 19% of flocks in the Residual Zones have been confirmed as infected, compared to about 5.7% in the Control Zones, 0.6% in the Protected Zones and less than 0.1% in the Free Zone.

Table 2.2. Cumulative number of flocks reported as infected between 1996 and 2002

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NSW</td>
<td>158</td>
<td>226</td>
<td>440</td>
<td>499</td>
<td>598</td>
<td>853</td>
<td>1,379</td>
<td>32,184</td>
</tr>
<tr>
<td>Vic.</td>
<td>33</td>
<td>66</td>
<td>97</td>
<td>175</td>
<td>204</td>
<td>226</td>
<td>257</td>
<td>30,000</td>
</tr>
<tr>
<td>SA</td>
<td>0</td>
<td>1</td>
<td>20</td>
<td>31</td>
<td>35</td>
<td>65</td>
<td>86</td>
<td>8,500</td>
</tr>
<tr>
<td>Tas.</td>
<td>6</td>
<td>8</td>
<td>19</td>
<td>21</td>
<td>23</td>
<td>28</td>
<td>38</td>
<td>2,050</td>
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<tr>
<td>ACT</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>85</td>
</tr>
<tr>
<td>Qld.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3,215</td>
</tr>
<tr>
<td>WA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>8,727</td>
</tr>
<tr>
<td>Total</td>
<td>197</td>
<td>301</td>
<td>578</td>
<td>728</td>
<td>863</td>
<td>1,176</td>
<td>1,765</td>
<td>84,671</td>
</tr>
</tbody>
</table>

* data as at 31 January 1998

2.4.5 Control measures

Regional control of OJD has relied mainly on regulatory measures of quarantine and movement controls. All Infected and Suspect flocks are subject to legally enforceable movement restrictions, with movement of stock for slaughter only or to flocks of the same or lower classification. Under Surveillance flocks may sell other than for slaughter, subject to disclosure of their classification and other relevant information. At the same time, movement restrictions between zones have been introduced to minimise the risk of spread from unidentified infected flocks into zones of a higher classification. These movement restrictions are based on the use of vendor declarations, including flock classification or testing history.
At the individual farm level, producers can take appropriate measures to prevent the introduction of OJD, including not grazing sheep in areas where they may be exposed to faeces from other sheep, such as paddocks that may be contaminated with faeces from adjoining properties, public roads and shared facilities. Other preventive measures include purchasing sheep only from properties classified in the SheepMAP and prevention of straying sheep. Although these measures are still largely untested, they will provide some protection against infection, particularly in areas where the risk of infection is low. In the Residual and Control Zones producers can also vaccinate to protect their stock from possible exposure to infection from nearby infected flocks. The effectiveness of vaccination, using a killed vaccine against *M. a paratuberculosis* (Gudair®), is currently being evaluated in three flocks in New South Wales (Windsor et al., 2002). Preliminary results from this trial have allowed registration of the vaccine for commercial sale and use in Australia. Vaccination now provides the main basis for control of infection in infected flocks and for management of the disease in the Residual Zone.

Owners of infected properties may choose to either live with the disease and adopt management strategies to try and minimise its effects, vaccinate to control the infection and its effects, or voluntarily attempt eradication by de-stocking the farm of sheep and goats. Current recommendations for eradication are to de-stock the farm of sheep and goats for a minimum of 15 months, including at least two summers. Short term grazing by susceptible sheep and goats during the eradication period is permitted, subject to conditions aimed at minimising the risk of recontamination. Alternatively, the land may be used for cropping, haymaking or grazing by cattle during this period (Anonymous, 1998a). Recent research has shown that de-stocking is not universally effective and that
infection had either persisted or been re-introduced on 11 of 26 properties evaluated two years post-restocking (Taylor et al., 2003).

### 2.5 Future directions

The NOJDP expires at the end of June 2004, although some of the initiatives, such as the SDRs and the SheepMAP, will continue beyond that date. The future of OJD control in Australia is therefore uncertain, although negotiations for a continuing national program have commenced. The NOJDP has fulfilled many of its objectives, particularly in relation to research and surveillance (Anonymous, 2003). Any future program is likely to build on the outcomes of the NOJDP, with an emphasis on continuing research and a shift from regulatory control of the disease to increased responsibility on producers for risk management for OJD (Allworth et al., 2003; Anonymous, 2003).
Part 2

Evaluation of screening tests for OJD
3. Evaluation of an absorbed ELISA and an agar-gel immuno-diffusion test for ovine paratuberculosis in sheep in Australia

3.1 Introduction

The main serological test used for flock-screening for ovine Johne’s disease in Australia has been the agar gel immuno-diffusion test (AGID). However, development of an ELISA for use in sheep would allow greater automation of testing procedures and enable rapid throughput for the increased volume of testing under the national program.

For flock testing, the flock-sensitivity achieved by a test depends on the animal-level sensitivity and specificity of the test, the number of sheep per flock that are screened and the prevalence of infection in infected flocks (Martin et al., 1992; Christensen and Gardner, 2000). Initially, sample sizes of 450 – 500 (depending on flock size) were required for serological testing in Australia, to provide a flock-sensitivity of 95% for an assumed sensitivity of serology of 30% and a within-flock prevalence of 2% (Anonymous, 2000b). Any sero-positive animals are further investigated by post-mortem and histopathology, so a high test specificity is also desirable, to reduce the number of false-positive reactors requiring further investigation.

Although serological tests for Johne’s disease in sheep were assumed to have a sensitivity of ~30% for that sample-size estimation, reported estimates of AGID and ELISA sensitivity vary from <10% in sheep with mild lesions to >90% in advanced clinical cases (Garcia Marin et al., 1991; Hilbink et al., 1994; Dubash et al., 1995;
Dubash et al., 1996; Perez et al., 1997). Where the AGID was evaluated in sheep that were randomly selected or where the whole flock was tested, the sensitivity was 24% (Huchzermeyer and Bastianello, 1991; Marshall et al., 1996), or 18% – 40% (Hope et al., 2000). Because of the variability between stages of disease, sensitivity is also likely to vary between flocks (depending on the frequency distribution of stages of disease in infected sheep in the flock). In contrast to the generally poor sensitivity, reported estimates of the specificity of AGID or ELISA tests for Johne’s disease in sheep are generally high when assessed in uninfected populations (98 – 100%) (Shulaw et al., 1993; Hilbink et al., 1994; Dubash et al., 1995; Hope et al., 2000).

Our aims were to estimate the sensitivity and specificity of an absorbed ELISA and an AGID for Johne’s disease in sheep and to evaluate the effect of flock of origin, lesion type and body-condition score on sensitivity estimates. Because of the potential for confounding of flock and condition-score effects by stage of disease, logistic multiple regression was used to examine the effects of each factor independently.

3.2 Materials and methods

3.2.1 Sample collection

Sensitivity and specificity of the tests were estimated using two separate sets of serum samples collected from sheep flocks in New South Wales. Samples used for the estimation of ELISA and AGID sensitivities were collected as part of an intensive study of six flocks from four known Johne’s disease-infected farms undertaken during 1995-96 (Marshall et al., 1996). All sheep in the six flocks (FLOCK; n = 1,379) were individually identified and their body-condition score (CS; on a scale of 1 = thin, 5 = fat; Table 3.1) determined by palpation of tissue cover over the backbone (O'Halloran,
All sheep were slaughtered at an abattoir and a 10-cm section of terminal ileum was collected and examined histologically. An animal was regarded as having lesions consistent with paratuberculosis (gold standard positive) if at least one aggregation of epithelioid macrophages was observed in the lamina propria of the terminal ileum (Perez et al., 1996; Cousins et al., 2001). Histological lesions were observed in tissue-sections from 224 sheep and were scored (HS) according to lesion severity and presence of acid-fast bacilli, using previously described categories (Perez et al., 1996) (Table 3.1). Histological lesion type (LT) was also categorised as either paucibacillary (HS = 1 – 4) or multibacillary (HS = 5), depending on the presence and abundance of acid-fast bacilli in histological sections (Table 3.1).

### Table 3.1. Variables included in logistic regression models to estimate ELISA and AGID sensitivity in 224 infected sheep from six flocks in New South Wales

<table>
<thead>
<tr>
<th>Variable name</th>
<th>Description</th>
<th>Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESULT</td>
<td>ELISA/AGID test result</td>
<td>0 = negative, 1 = positive</td>
</tr>
<tr>
<td>FLOCK</td>
<td>Flock of origin</td>
<td>1, 2, 3, 4, 5, 6 = Flock 1, Flock 2, Flock 3, Flock 4, Flock 5, Flock 6</td>
</tr>
<tr>
<td>CS</td>
<td>Condition Score (measured 110mm from the midline over the 12th rib)</td>
<td>1 = Individual ribs felt very easily; cannot feel any tissue over the ribs, 2 = Individual ribs easily felt, but some tissue present, 3 = Individual ribs can still be felt, but can feel tissue, 4 = Can just feel ribs and fluid movement of tissue, 5 = Ribs barely felt; tissue movement very fluid (There were no sheep in this study with CS = 5)</td>
</tr>
<tr>
<td>HS</td>
<td>Histological lesion score</td>
<td>1 = mild lesion, no acid-fast bacilli observed – Type 1 from Perez et al (1997), 2 = mild lesion, few acid-fast bacilli observed – Type 2 from Perez et al (1997), 3 = moderate-severe lesion, no acid-fast bacilli observed – Type 3c from Perez et al (1997), 4 = moderate-severe lesion, few acid-fast bacilli observed – Type 3a from Perez et al (1997), 5 = moderate-severe lesion, many acid-fast bacilli observed – Type 3b from Perez et al (1997)</td>
</tr>
<tr>
<td>LT</td>
<td>Lesion type</td>
<td>paucibacillary = lesions with HS = 1 – 4, multibacillary = lesions with HS = 5</td>
</tr>
</tbody>
</table>
Serum samples used for the estimation of the specificities of both tests were archival samples collected from sheep on properties believed to be free of *M. paratuberculosis* infection at the time of collection, and still believed to be free. All sheep in this analysis were believed to be free of infection, based on flock history and location. However, no additional tests (such as histology or faecal culture) were possible to confirm the infection-free status of these sheep. For the ELISA, 1,748 serum samples from 12 flocks were used to estimate specificity. Of these 1,748 samples, 1,584 (from 10 flocks) were collected during 1988 – 1991, when the geographic distribution of ovine Johne’s disease in New South Wales was thought to be quite restricted. Also, 1,255 of the samples (from five flocks) were from areas of western New South Wales where Johne’s disease is not known to occur. All 1,748 samples were used for the evaluation, because the samples were available at no cost, the sample size was large enough to ensure reasonably precise estimates of specificity and the samples provided a reasonable cross-section of sheep-age and geographic location. Because there was insufficient serum available in some samples, only 1,387 of these samples (from nine flocks) also were used for estimating AGID specificity.

### 3.2.2 ELISA methodology

The method was based on that of Yokomizo et al. (Yokomizo et al., 1983; Yokomizo et al., 1985). The ELISA was redesigned for use in sheep and tested at the Regional Veterinary Laboratory, Orange and Elizabeth Macarthur Agricultural Institute, Camden, Australia. The test was approved for use in Australia following preliminary evaluation of sensitivity and specificity (Marshall and Whittington, 1997), and these data were made available for the present analysis. The methodology has not been described elsewhere. Briefly, optimal reagent concentrations and incubation times were determined by titration with conditions resulting in greatest signal : noise ratio (positive
control serum: negative control serum) being selected. Test sera diluted 1:200 in phosphate-buffered saline (PBS) with 0.05% Tween 20 and 0.1% gelatin (PBSTG) were absorbed with *M phlei* whole-cells overnight at 4°C with constant rotation, then centrifuged at 1500 rpm to remove particulate matter. Flat-bottomed ELISA plates were coated overnight at 5°C with antigen derived from mechanically disrupted *M a paratuberculosis* strain 316V (IS900 BstEII RFLP cattle strain). After washing plates four times with PBS containing 0.05% v/v Tween 20 (PBST) in an automatic 96-well ELISA plate washer, aliquots of 100 ul of diluted test serum were added to each well and incubated for 90 min at room temperature (RT). After washing plates, 100 ul of affinity-purified rabbit anti-sheep IgG (H+L chain) conjugated to horseradish peroxidase (KPL Laboratories) diluted 1:3000 in PBSTG was added to each well and incubated for 90 min at RT. After washing, 100 ul of chromogenic substrate (2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulphonic acid)) (ABTS) with hydrogen peroxide was added to each well and incubated at RT for 20 min. The reaction was stopped using 50 ul 0.01% sodium azide in citrate-phosphate buffer and optical density (OD) was read at 414 nm after shaking the plate for 10 seconds at 100 RPM. Each test serum was evaluated in two adjacent wells. Negative control sera were included in 6 wells on each plate. A high-positive and a low-positive control serum were included in paired wells on each plate. Plates were rejected and testing repeated if either the average of the negative control sera or the average of the high-positive and low-positive control sera deviated by more than 20% from the long-term averages. Results were expressed as an ELISA ratio: the mean OD of the test serum divided by the mean OD of the 6 wells containing the negative control serum.
3.2.3 AGID methodology

The method was based on those of Merkal et al., (1968) and Goudswaard and Terporten-Pastoors, (1972), and was described as the ovine gel-test by Whittington et al., (2003).

3.2.4 Sensitivity of ELISA and AGID

ELISA sensitivity was estimated for a range of ELISA ratios, including cut-point ratios corresponding to specificities of 95% and 99%. Sensitivity at each cut-point was calculated as the percentage of samples that had an ELISA result greater than or equal to the cut-point. AGID sensitivity was calculated as the percentage of samples that had a positive AGID result. Exact binomial 95% confidence limits for sensitivity estimates were calculated using Epi Info v6.04. The chi-squared test (two-tailed, $\alpha = 0.05$) was used to test for significant univariate associations between FLOCK and CS, HS and LT, between test result and FLOCK, CS, HS and LT and between CS and LT. $P$-values for HS and LT comparisons were adjusted according to Bonferroni’s rule for multiple comparison’s. One flock was excluded from univariate comparisons with FLOCK because the small numbers of sheep in this flock ($n=8$) rendered the chi-squared test invalid if it was included.

McNemar’s test (two-tailed, $\alpha = 0.05$) was used to test for significant differences in discordant results between the AGID and the ELISA at both cut-points and sensitivity covariances were calculated for the AGID and ELISA at each cut-point (Gardner et al., 2000). The kappa statistic (two-tailed, $\alpha = 0.05$) was used to evaluate the level of agreement between AGID and ELISA beyond chance.
3.2.5 Multivariable analysis of test result for ELISA and AGID

Because of possible confounding by HS, multiple logistic regression was used to further evaluate the effects of FLOCK, CS and HS on the odds of a positive test result for the ELISA and AGID. For these analyses, the dichotomized test result (RESULT) for the ELISA or AGID was the dependent variable, CS and HS were independent fixed-effect variables and FLOCK was included in the model as a random-effect variable. Separate analyses were done for ELISA cut-point ratios corresponding to specificities of 95% and 99%, and for the AGID. An interaction term for CS*HS was tested in each of the models, but was not included because multiple collinearities made the models unstable. For each analysis, the fit of the original model also was compared with alternative regression models in which CS and HS in turn were excluded from the model. The likelihood-ratio test was used to test for statistical significance of any differences between the original and alternative models and for the significance of the random effect variable (two-tailed, $\alpha = 0.05$). Stata (Stata Corporation, Texas) was used for the logistic-regression analysis, with $P < 0.05$ regarded as significant.

3.2.6 Specificity of ELISA and AGID

The ELISA specificity was estimated for a range of ELISA ratio cut-points, assuming that any positive results recorded were false positives. For any selected cut-point, specificity was estimated as the percentage of samples that had an ELISA ratio less than the nominated cut-point. Cut-point ELISA ratios were determined to provide estimated specificities of 95% and 99%. Differences in specificity between sheep < 1 year old and $\geq$ 1 year old at each of the cut-points were tested for statistical significance using a chi-squared test (two-tailed, $\alpha = 0.05$). The AGID specificity was estimated as the percentage of samples that yielded a negative AGID test result. Exact binomial 95% confidence limits for specificity estimates were calculated using Epi Info v6.04.
3.3 Results

3.3.1 Sensitivity of ELISA and AGID

The numbers of infected sheep and the characteristics of each flock are summarised in Table 3.2. The histological prevalence and percentages of animals in each category of CS, HS and LT all varied significantly between flocks. The percentage of sheep with multibacillary lesions varied significantly between CS categories, from 76% for CS = 1, to 52%, 18% and 22% for CS = 2, 3 and 4 respectively ($\chi^2 = 34.3, df = 3, P < 0.001$).

ELISA ratios in the 224 sheep with histological lesions ranged from 1.0 to 18.0, with cut-point ratios of 2.4 and 3.6 corresponding to specificities of 95% and 99% respectively (see Section 3.3.3). ELISA sensitivities across all sheep were 41.5% and 21.9% for ELISA cut-points of 2.4 (ELISA-2.4) and 3.6 (ELISA-3.6), respectively and 24.6% for the AGID (Table 3.3, Figure 3.1). Sensitivities of ELISA and AGID varied significantly between flocks, and according to CS, HS and LT (Table 3.3). The AGID and ELISA both were more sensitive in sheep with multibacillary lesions than in those with paucibacillary lesions. For each test and cut-point, sensitivity was similar for HS categories of 1, 2, 3 and 4, except that for both ELISA cut-points sensitivities for HS = 3 were more than twice the value for the other categories. The ELISA and AGID also were more sensitive in poor condition (CS $\leq$ 2) sheep than in those in better condition (CS $>$ 2) ($\chi^2 = 18.8, df = 1, P < 0.001$ for ELISA-2.4; $\chi^2 = 22.8, df = 1, P < 0.001$ for ELISA-3.6 and $\chi^2 = 63, df = 1, P < 0.001$ for AGID).

ELISA sensitivity at a cut-point of 2.4 was significantly higher than that for the AGID ($\chi^2 = 33, df = 1, P < 0.001$, kappa = 0.49, SE(kappa) = 0.62, $P$(kappa) = <0.001) but not
Table 3.2. The number of sheep, their age and sex, the number and percentage with histological lesions of paratuberculosis, and the percentages in each HS, LT and CS category for each of six flocks of merino sheep, used for the estimation of the sensitivity of ELISA and AGID for the detection of paratuberculosis in sheep in New South Wales

<table>
<thead>
<tr>
<th>Flock</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number in flock</td>
<td>272</td>
<td>73</td>
<td>93</td>
<td>308</td>
<td>441</td>
<td>192</td>
<td>1379</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>5</td>
<td>Various</td>
<td>Various</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Wethers</td>
<td>Various</td>
<td>Mixed</td>
<td>Ewes</td>
<td>Wethers</td>
<td>Wethers</td>
<td></td>
</tr>
<tr>
<td>Number with lesions</td>
<td>30</td>
<td>17</td>
<td>8</td>
<td>38</td>
<td>87</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Histological prevalence (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0</td>
<td>23.3</td>
<td>8.6</td>
<td>12.3</td>
<td>19.7</td>
<td>22.3</td>
<td>16.2</td>
</tr>
</tbody>
</table>

Percentage<sup>b</sup> of flock in each histology score or lesion type

| HS = 1 | 20.0 | 17.6 | 12.5 | 28.9 | 23.0 | 13.6 | 21.0 |
| HS = 2 | 3.3 | 5.9 | 0.0 | 7.9 | 12.6 | 2.3 | 7.6 |
| HS = 3 | 30.0 | 41.2 | 0.0 | 10.5 | 24.1 | 13.6 | 21.0 |
| HS = 4 | 6.7 | 23.5 | 25.0 | 21.1 | 26.4 | 25.0 | 22.3 |
| HS = 5 | 40.0 | 11.8 | 62.5 | 31.6 | 13.8 | 45.5 | 28.1 |

Percentage<sup>c</sup> of flock with each lesion type

| LT = Paucibacillary | 60.0 | 88.2 | 37.5 | 68.4 | 86.2 | 54.5 | 71.9 |
| LT = Multibacillary | 40.0 | 11.8 | 62.5 | 31.6 | 13.8 | 45.5 | 28.1 |

Percentage<sup>d</sup> of flock in each condition score

| CS = 1 | 23.3 | 0.0 | 25.0 | 13.2 | 0.0 | 6.8 | 7.6 |
| CS = 2 | 23.3 | 11.8 | 50.0 | 10.5 | 9.2 | 9.1 | 12.9 |
| CS = 3 | 33.3 | 35.3 | 25.0 | 42.1 | 72.4 | 52.3 | 53.6 |
| CS = 4 | 20.0 | 52.9 | 0.0 | 34.2 | 18.4 | 31.8 | 25.9 |

<sup>a</sup> Differences in histological prevalence between flocks were significant ($\chi^2 = 26$, df = 5, $P < 0.001$)

<sup>b</sup> Differences in distribution of HS between flocks (excluding Flock 3) were significant ($\chi^2 = 34$, df = 16, $P = 0.01$)

<sup>c</sup> Differences in LT between flocks (excluding Flock 3) were significant ($\chi^2 = 25$, df = 4, $P < 0.001$)

<sup>d</sup> Differences in distribution of CS between flocks (excluding Flock 3) were significant ($\chi^2 = 43$, df = 12, $P < 0.001$)
at a cut-point of 3.6 ($\chi^2 = 0.7$, df = 1, $P = 0.4$, kappa = 0.55, SE(kappa) = 0.67, $P(kappa) = <0.001$). Although there was significant agreement beyond chance with AGID results, for both ELISA cut-points, there were significantly more discordant results in the ELISA+, AGID– direction than in the opposite direction for ELISA-2.4 (Table 3.4). Covariances for AGID and ELISA sensitivities were 0.11 (78% of maximum) for ELISA-2.4 and 0.10 (59% of maximum) for ELISA-3.6.

Figure 3.1. Chart of estimated sensitivity and specificity of an absorbed ELISA for ovine Johne’s disease, for ELISA ratio values from 0.7 to 4.5. Sensitivity was estimated in 224 sheep from six infected flocks New South Wales and specificity was estimated in 1748 sheep of mixed ages, as well as in 400 sheep < 1 year old and 997 sheep $\geq$ 1 year, from 12 presumed uninfected flocks in New South Wales.
3.3.2 Multivariable analysis of ELISA and AGID results

Summary results of the multivariable analyses are shown in Table 3.5. For all analyses, HS=5 had significantly higher odds of a positive result than HS=1, while HS = 3 was significant for the ELISA-2.4 and close to significant \( P = 0.067 \) for the ELISA-3.6, but not for the AGID. CS categories of 2, 3 and 4 all had significantly lower odds of a positive result than CS=1 for the ELISA-3.6 and the AGID, but not for the ELISA-2.4, although CS = 3 was significant \( P = 0.047 \) and CS = 4 was close to significance \( P = 0.064 \) for the latter cut-point.

Likelihood ratios for the regression models were 31.5 and 36.2 for ELISA-2.4 and ELISA-3.6 respectively and 76.4 for the AGID (df = 7, \( P < 0.001 \) for all three models). The deviance for the ELISA models with cut-points of 2.4 and 3.6 and for the AGID model were 259.3, 190.7 and 153.3 respectively (df = 216), indicating reasonable fit of the models, except for the ELISA-2.4. Removal of either CS or HS from the models resulted in a significant reduction in the likelihood ratio, for both independent variables \( P < 0.05 \) for ELISA at both cut-points and \( P < 0.001 \) for the AGID), except that removal of CS resulted in a non-significant change for the ELISA-2.4 (LR = 7.2, df = 3, \( P = 0.066 \)). CS was retained in the latter model because its effect was close to significant, and because it was an important factor in the other models. The random effect due to FLOCK was significant for the AGID \( \chi^2 = 7.45, \) df = 1, \( P = 0.006 \)\), but not for the ELISA-2.4 \( \chi^2 = 3.16, \) df = 1, \( P = 0.076 \)\) or ELISA-3.6 \( \chi^2 = 0.88, \) df = 1, \( P = 0.35 \)\). FLOCK was retained in the models for both ELISA cut-points, despite the lack of significance, because of its significance in the AGID model, and because herd or flock effects are recognized as potentially important confounders (Curtis et al., 1993).
Table 3.3. Estimated sensitivity and 95% confidence intervals for an ovine Johne’s disease ELISA at two cut-point ELISA ratios and for the AGID in 224 infected sheep from six flocks in New South Wales, Australia

<table>
<thead>
<tr>
<th></th>
<th>ELISA cut-point = 2.4</th>
<th></th>
<th>ELISA cut-point = 3.6</th>
<th></th>
<th>AGID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Se (%)</td>
<td>95% CI (%)</td>
<td>n</td>
<td>Se (%)</td>
</tr>
<tr>
<td><strong>FLOCK</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>25.3</td>
<td>16.6, 35.7</td>
<td>10.3</td>
<td>4.8, 18.7</td>
<td>9.2</td>
</tr>
<tr>
<td>Maximum</td>
<td>73.3</td>
<td>54.1, 87.7</td>
<td>47.1</td>
<td>23.0, 72.2</td>
<td>62.5</td>
</tr>
<tr>
<td>Weighted average</td>
<td>41.5</td>
<td>35.0, 48.3</td>
<td>21.9</td>
<td>16.6, 27.9</td>
<td>24.6</td>
</tr>
<tr>
<td>across all flocks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Histology score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS = 1</td>
<td>47</td>
<td>25.5</td>
<td>13.9, 40.3</td>
<td>10.6</td>
<td>3.5, 23.1</td>
</tr>
<tr>
<td>HS = 2</td>
<td>17</td>
<td>23.5</td>
<td>6.8, 49.9</td>
<td>11.8</td>
<td>1.5, 36.4</td>
</tr>
<tr>
<td>HS = 3</td>
<td>47</td>
<td>51.1</td>
<td>36.1, 65.9</td>
<td>27.7</td>
<td>15.6, 42.6</td>
</tr>
<tr>
<td>HS = 4</td>
<td>50</td>
<td>24.0</td>
<td>13.1, 38.2</td>
<td>8.0</td>
<td>2.2, 19.2</td>
</tr>
<tr>
<td>HS = 5</td>
<td>63</td>
<td>65.1</td>
<td>52.0, 76.7</td>
<td>39.7</td>
<td>27.6, 52.8</td>
</tr>
<tr>
<td><strong>Lesion type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT = Paucibacillary</td>
<td>161</td>
<td>32.3</td>
<td>25.2, 40.1</td>
<td>14.9</td>
<td>9.8, 21.4</td>
</tr>
<tr>
<td>LT = Multibacillary</td>
<td>63</td>
<td>65.1</td>
<td>52.0, 76.7</td>
<td>39.7</td>
<td>27.6, 52.8</td>
</tr>
<tr>
<td><strong>Condition score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS = 1</td>
<td>17</td>
<td>82.4</td>
<td>56.6, 96.2</td>
<td>76.5</td>
<td>50.1, 93.2</td>
</tr>
<tr>
<td>CS = 2</td>
<td>29</td>
<td>62.1</td>
<td>42.3, 79.3</td>
<td>31.0</td>
<td>15.3, 50.8</td>
</tr>
<tr>
<td>CS = 3</td>
<td>120</td>
<td>32.5</td>
<td>24.2, 41.7</td>
<td>12.5</td>
<td>7.2, 19.8</td>
</tr>
<tr>
<td>CS = 4</td>
<td>58</td>
<td>37.9</td>
<td>25.5, 51.6</td>
<td>20.7</td>
<td>11.2, 33.4</td>
</tr>
</tbody>
</table>

*a* differences between categories were significant at both ELISA cut-points and for the AGID, for FLOCK, HS, LT and CS ($P < 0.001$ for all tests)

*b* sensitivities of ELISA at a cut-point of 2.4 and AGID were significantly different ($P < 0.001$)
Table 3.4. Paired comparison of AGID result with ELISA result at cut-point ratios of 2.4 and 3.6 in 224 infected sheep from six flocks in New South Wales, Australia

<table>
<thead>
<tr>
<th>AGID</th>
<th>ELISA 2.4&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ELISA 3.6&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>48</td>
<td>34</td>
<td>55</td>
</tr>
<tr>
<td>–</td>
<td>45</td>
<td>124</td>
<td>169</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>131</td>
<td>224</td>
</tr>
</tbody>
</table>

<sup>a</sup> $\chi^2 = 33$, df = 1, $P < 0.001$; <sup>b</sup> $\chi^2 = 0.7$, df = 1, $P = 0.4$

Table 3.5. Logistic regression models of the effect of CS and LT on ELISA result at cut–point ratios of 2.4 and 3.6 and on AGID result, with FLOCK included as a random effect, in 224 sheep with histological lesions of paratuberculosis from six infected flocks in New South Wales

<table>
<thead>
<tr>
<th>Test Variable</th>
<th>Category</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA-2.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.59</td>
<td>0.12, 2.82</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.23</td>
<td>0.05, 0.98</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.25</td>
<td>0.06, 1.08</td>
<td>0.064</td>
</tr>
<tr>
<td>HS</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.97</td>
<td>0.25, 3.82</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.16</td>
<td>1.26, 7.91</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.97</td>
<td>0.37, 2.56</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.98</td>
<td>1.59, 9.92</td>
<td>0.003</td>
</tr>
<tr>
<td>ELISA-3.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.18</td>
<td>0.04, 0.77</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.07</td>
<td>0.02, 0.28</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.12</td>
<td>0.03, 0.48</td>
<td>0.003</td>
</tr>
<tr>
<td>HS</td>
<td>1</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.93</td>
<td>0.14, 6.04</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.02</td>
<td>0.92, 9.85</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.67</td>
<td>0.16, 2.8</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.12</td>
<td>0.98, 9.92</td>
<td>0.054</td>
</tr>
<tr>
<td>AGID</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.070</td>
<td>0.01, 0.94</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.017</td>
<td>0.001, 0.21</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.007</td>
<td>0.0005, 0.1</td>
<td>0.000</td>
</tr>
<tr>
<td>HS</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.32</td>
<td>0.02, 5.28</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.12</td>
<td>0.26, 4.73</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.14</td>
<td>0.27, 4.83</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9.28</td>
<td>2.6, 33.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>
3.3.3 Specificity of ELISA and AGID

The age of sheep used for the specificity evaluation ranged from < 4 months to > 6 years, and their ELISA ratios ranged from 0.7 to 7.3 (median 1.5). There were 400 samples from sheep < 1 year old, 997 from sheep ≥ 1 year old and 351 where age was not recorded. The median ELISA ratio for sheep ≥ 1 year old (ER = 1.5) was significantly higher than that for sheep < 1 year of age (ER = 1.1; Mann-Whitney U test: $\chi^2 = 326, \text{df} = 1, P < 0.001$).

ELISA specificity was estimated for cut-point ratios ranging from 0.7 to 7.3 for all sheep sampled, and separately for sheep < 1 year old and ≥ 1 year old (Figure 3.1). Cut-point ratios of 2.4 and 3.6 gave specificities of 95% and 99% respectively for all sheep and for sheep ≥ 1 year old, while specificity was higher in the younger sheep at the same cut-point (Table 3.6, Figure 3.1). The difference in specificity between age groups was significant at a cut-point ratio of 2.4 ($\chi^2 = 15, \text{df} = 1, P < 0.001$), but not at a cut-point of 3.6 ($\chi^2 = 3.2, \text{df} = 1, P = 0.08$). All 1387 samples tested with the AGID were negative, so the estimated specificity of the AGID was 100% (95% CI: 99.7, 100.0%). Because AGID specificity was 100% in this analysis, specificity covariances were zero.

### Table 3.6. Estimated specificity of an ovine Johne’s disease ELISA for cut-point ratios of 2.4 and 3.6 in archival samples collected from 1748 sheep from 12 presumed uninfected flocks in New South Wales, Australia between 198 and 1996, and of an AGID for a subset of 1387 sheep from nine of the 12 flocks

<table>
<thead>
<tr>
<th>Test</th>
<th>Number tested</th>
<th>Number positive</th>
<th>Sp (%)</th>
<th>95% CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA-2.4</td>
<td>1748</td>
<td>77</td>
<td>95</td>
<td>93.4, 95.6</td>
</tr>
<tr>
<td>ELISA-3.6</td>
<td>1748</td>
<td>18</td>
<td>99</td>
<td>98.4, 99.4</td>
</tr>
<tr>
<td>AGID</td>
<td>1387</td>
<td>0</td>
<td>100</td>
<td>99.7, 100</td>
</tr>
</tbody>
</table>
3.4 Discussion

3.4.1 Test sensitivity varies between flocks

These results confirm that AGID and ELISA sensitivities vary significantly between flocks, and according to histological lesion type and body condition-score of infected sheep, probably indicating variation with stage of disease. However, even after controlling for the effects of lesion type and condition score, flock of origin still had a significant effect on sensitivity for the AGID, although not for the ELISA. The sources of this additional flock-effect could not be determined from this study, but might include age, nutrition, the occurrence of other diseases such as internal parasitism, or variations in stage of disease that were not detected because of the histological method used. One possible reason that the flock-effect was significant for the AGID but not the ELISAs might have been because the ELISA detected more good-condition sheep (CS = 4) and sheep with paucibacillary lesions (particularly HS = 2 and HS = 3) than did the AGID.

3.4.3 Test sensitivity varies with histological lesion type

Sensitivity in sheep with paucibacillary lesions generally is low because the development of a humoral response usually is associated with proliferation of \textit{M a paratuberculosis} and the presence of larger numbers of organisms in lesions, as observed in sheep with multibacillary lesions (Perez et al., 1997; Clarke and Little, 1996). This relationship also was observed in this study, although ELISA sensitivity was higher in sheep with HS = 3 (equivalent to type 3c lesions from Perez et al (1997)) than in those with HS = 1, 2 or 4. The reason for this discrepancy is unknown, but could
also be due to misclassification of some lesions, although the difference was not apparent for the AGID.

3.4.3 Test sensitivity varies with condition score

A strong association between condition score and lesion type also was observed, with a higher percentage of multibacillary cases in poorer-condition sheep, and predominantly paucibacillary cases in good-condition sheep. This suggests that the apparent association between condition score and sensitivity might have been due to confounding by lesion type. However, condition score still had a significant association with sensitivity after controlling for the effects of flock and lesion type. This might have been due to misclassification of some multibacillary cases as paucibacillary, because of the limitations of histological examination of sections from a single segment of ileum. If this were the case, then sensitivities would have been overestimated in sheep with true paucibacillary lesions.

The association between condition score and sensitivity provides a possible mechanism for improving test performance in the field, by targeted testing of poor condition (CS≤2) sheep, rather than using systematic sampling as is currently the case (Marshall et al., 1996). For example, AGID sensitivity in sheep with CS≥3 was about 13%, compared to 70% for sheep with CS≤2. Thus, targeting of poor-condition sheep would require smaller sample sizes and still would provide high confidence of detecting infection — whereas systematic sampling or testing of predominantly good-condition sheep would require much-larger sample sizes to achieve the same flock-sensitivity. However, in flocks with few (if any) poor-condition sheep, it would be necessary to test much-larger numbers of good-condition sheep, (or accept a lower flock-sensitivity in such flocks on
the assumption that they are less likely to be infected if there are few poor-condition sheep).

### 3.4.4 Potential biases from an imperfect gold standard

An important source of possible bias in this study was the assumption that histology was perfectly sensitive and specific. In reality, this assumption is unlikely to be correct, and both sensitivity and specificity are likely to be < 100%. Imperfect sensitivity of histology could have occurred because histology included examination of sections from only a single segment of ileum (rather than from a wider range of tissues as is usually the situation in investigation of suspect cases) (Cousins et al., 2001). Therefore, because the estimates presented here were not adjusted for the imperfect sensitivity of histology, they are likely to over-estimate the true values of sensitivity to an unknown extent (Staquet et al., 1981). This over-estimation might be greater for sheep with early, paucibacillary lesions, which are the sheep least likely to be detected by histology; the difference in sensitivity between sheep with paucibacillary and multibacillary lesions might be even greater than reported here. Alternative methods that do not depend on comparison with a “gold standard” could have been used to adjust for the imperfect sensitivity of histology (Hui and Walter, 1980; Enøe et al., 2000; Johnson et al., 2001), but estimates would have been affected by the variability of sensitivity between populations and likely correlations between test sensitivities. Also, such methods would not have allowed for full investigation of effects of flock, lesion type and condition score on test sensitivity.

Conversely, imperfect specificity of histology could have occurred because of the assumption that animals having histological lesions without demonstrable acid-fast
bacilli were infected at the time of slaughter. These lesions were assumed to be due to paratuberculosis — but could be residual lesions in animals that have eliminated the infection. If these sheep were not currently infected, any sensitivity estimates including sheep in these categories (HS = 1 or 3) would under-estimate the true sensitivity. However, the regression coefficients for HS = 2 – 4 were not significantly different from zero, except for HS = 3 at an ELISA cut-point of 2.4. This finding supports the hypothesis that sensitivities for HS = 2 – 4 did not differ significantly from that for the reference category (HS = 1), and the assumption that these sheep were truly infected is likely to be correct. This assumption also has been used for the classification of paratuberculosis lesions in other studies (Perez et al., 1996; Perez et al., 1997). In one study, the presence of *M. paratuberculosis* was confirmed by tissue culture and/or polymerase chain reaction for the IS900 sequence in 67% of such cases (Perez et al., 1994).

### 3.4.5 Multivariable analyses

In the logistic regression analyses, the final regression models for the AGID and the ELISA at a cut-point of 3.6 appeared to fit well, with the deviance values less than the degrees of freedom for both models. However, for the ELISA at a cut-point of 2.4 the deviance was considerably greater than the degrees of freedom, suggesting that there was still additional extra-binomial variation that was not adequately accounted for by the flock effect. This could have been due to non-specific reactions in some infected sheep, associated with the lower specificity of the ELISA at this cut-point, or to some other factor that was not recorded. The poorer fit of this model could also have accounted for the reduced significance of CS in this model, compared to the AGID and other ELISA models.
3.5 Conclusion

The estimated test specificities were 99% and 95% for the ELISA at cut-point ratios of 3.6 and 2.4 respectively, and 100% for the AGID. Corresponding sensitivities were 21.9%, 41.5% and 24.6%, respectively. Sensitivity varied significantly between flocks and according to condition score and lesion type of infected sheep. Because these estimates were not adjusted for the imperfect sensitivity of histology, they are likely to over-estimate the true values to an unknown extent.

Considering the very large sample sizes required for serological flock-screening in Australia and its lower specificity than the AGID, the ELISA is likely to result in an unacceptably high number of false-positive reactors requiring further testing to clarify their infection status. A higher ELISA sensitivity could be achieved using the lower cut-point ratio of 2.4, but this would result in even more false-positive reactors, and is therefore not acceptable. For this reason the AGID is used routinely and the ELISA seldom used in the national ovine Johne’s disease control program in Australia.
4. Sensitivity and specificity of pooled faecal culture and serology as flock-screening tests for detection of ovine paratuberculosis in Australia

4.1 Introduction

Traditionally in Australia, testing for paratuberculosis relied on serology using the agar-gel immunodiffusion test (AGID), with any reactors submitted to postmortem histological examination to confirm or exclude infection (Whittington and Sergeant, 2001; Sergeant, 2001). It is only recently that techniques have been developed to allow reliable culture of ovine strains of *M. a paratuberculosis* from tissues and faeces of infected animals (Whittington et al., 1998; Whittington et al., 1999). This technology was extended by the development of pooled faecal culture (PFC) as a flock-screening test for paratuberculosis (Whittington et al., 2000a). PFC has been used routinely for OJD surveillance in New South Wales since 1999 (see Chapter 2).

Whittington et al. (2000a) described the development of PFC and its comparison with the existing strategy of serology followed by histopathology on reactors. In the original evaluation, PFC appeared more sensitive than serology, but quantitative estimates of flock-sensitivity and flock-specificity were not made (Whittington et al., 2000a). Because of the pooling of samples, PFC also provides a much-cheaper test for screening at the flock level (Whittington et al., 2000a).
Although PFC is regarded as 100% specific (at least at the flock level), it is essential that this is confirmed in field usage of the test to provide confidence in the performance of the test. Similarly, quantitative estimates of the flock-sensitivity of PFC are required to support its use as the preferred (that is, most-sensitive) screening test for OJD in Australia and to allow determination of appropriate sample sizes for testing programs.

Estimation of the sensitivity and specificity of flock-screening tests for paratuberculosis is further complicated by the fact that there is not a reliable ‘gold standard’ for flock status against which to compare results and evaluate performance of the test. Although both PFC and serology/histology are highly specific flock tests, both have imperfect sensitivity (Stehman, 1996; Whittington et al., 2000a; Whittington and Sergeant, 2001). Thus, use of traditional methods dependent on a gold standard for determining flock-sensitivity and flock-specificity may result in estimates that are importantly biased (Staquet et al., 1981; Greiner and Gardner, 2000; Enøe et al., 2000). Enøe et al. (2000) recently reviewed techniques for estimating test sensitivity and specificity in the absence of a gold standard. Although these techniques have been developed mainly for use with tests applied to individuals, the methods are equally applicable to flock-level tests such as PFC and serology.

The aims of this study were to evaluate the flock-sensitivity for both tests, to compare test performance in flocks with high and low prevalence, to confirm the adequacy or otherwise of current sample sizes for detection of OJD using these tests and to estimate the flock-level specificity of PFC. Because there was no gold standard available for flock-infection status, several non-gold-standard methods were used for this evaluation.
4.2 Methods

4.2.1 Estimation of flock-specificity

*Estimation of flock-specificity of pooled faecal culture*

The flock-specificity of PFC was estimated using the results of surveillance and assurance testing in areas of New South Wales where OJD was thought to be at a low or zero flock-prevalence, using a method adapted from Seiler (1979). Results from routine use of PFC in New South Wales between 1 January 1999 and 31 October 2000 were used for this analysis. Only submissions for which culture had been completed and which were from districts with <20 known-infected flocks (equivalent to a prevalence of known-infected flocks of <~2%) at 30 September 2000 were included in the analysis. For all positive results, the flocks of origin were further investigated either by serology or by repeated faecal culture to determine whether or not infection could be confirmed. Any cases where infection could not be confirmed were regarded as false-positive results. The minimum flock-specificity of PFC in the field was then estimated as 100 minus the percentage of false-positive submissions.

4.2.2 Estimation of flock-sensitivity

*Flock selection and testing*

Whittington et al. (2000a) described the selection and testing of the flocks used to estimate the flock-sensitivity of PFC and serology. These flocks were tested between April and December 1998 as part of an ongoing surveillance program for OJD in Australia. Briefly, flocks were selected for testing either because there was some suspicion of infection due to tracing of sheep movements to/from an infected flock, or to demonstrate a flock’s low-risk status for entry to the market-assurance program.
flocks with no evidence of disease or an expected low prevalence, sample sizes were chosen to provide 95% confidence of detecting a prevalence of 2%, assuming an animal-level test sensitivity of 30% for the AGID. In these flocks, selection of individual sheep for testing was carried out in a systematic manner across all adult sheep in the flock. Where the disease was likely to be at a higher prevalence, smaller sample sizes were used, and selection of sheep for testing was biased to include the animals most likely to be infected (older or poorer-condition sheep, or those introduced from known-infected flocks). These sample sizes and selection strategies were based on current recommendations for surveillance and assurance testing for OJD in Australia (Anonymous, 1998a; Anonymous, 2000b). In each flock, the selected sheep were tested using both the AGID and PFC, and any seropositive animals were investigated further by postmortem examination and histology to determine their infection status.

Single faecal pellets were collected from the rectum of each sheep and aggregated into pools of up to 50 pellets for PFC testing. In flocks where the sample size was not a multiple of 50, one pool contained pellets from less than 50 sheep. All pooled samples were homogenised, decontaminated and cultured for \textit{M. paratuberculosis} as described by Whittington et al. (2000a). A flock was regarded as positive for PFC if \textit{M. paratuberculosis} was isolated either in primary BACTEC medium, and/or in primary or secondary culture on solid medium from one or more pools. All isolates were confirmed as \textit{M. paratuberculosis} by positive PCR for the IS\textsubscript{900} sequence and restriction-endonuclease analysis (REA). An inconclusive PFC result was recorded if one or more pools were positive on PCR but insufficient reaction product was available to undertake REA. A flock was positive to serology/histology if one or more animals were identified
with histological lesions consistent with paratuberculosis and in which acid-fast bacilli were observed. An inconclusive flock was one in which one or more animals had histological lesions suggestive of paratuberculosis (presence of giant cells and/or accumulations of three or more epithelioid macrophages), but in which no acid-fast organisms could be identified. Flocks in which there either were no seroreactors, or in which all reactors investigated were negative on histology were regarded as negative.

**Within-flock prevalence estimation using AGID**

Within-flock seroprevalence and true prevalence were estimated for all flocks that were positive to one or both flock tests. Seroprevalence was calculated as the percentage of sheep tested in each flock that were seropositive. The within-flock true prevalence was estimated by adjusting the seroprevalence for an assumed animal-level sensitivity and specificity for the AGID of 30% and 100% respectively (Rogan and Gladen, 1978). This approach could not be used in seronegative flocks, because it yields a zero prevalence. In these flocks, the true prevalence was estimated as the upper 50% confidence limit of the estimated prevalence for zero positives from the given sample size, divided by the assumed sensitivity of the AGID (30%). An upper limit of 50% was chosen instead of 95% because an estimate of the ‘average’ within-flock prevalence was required, rather than an estimate of the likely maximum prevalence. An average sensitivity of 30% was used, because this is the assumed value used in sample size calculations for surveillance and assurance testing in Australia.

Flocks were categorised as low or high prevalence if the estimated true prevalence was <2% or ≥2%, respectively, and by sample size as <350 or ≥350 sheep sampled for
serology. The cut-off sample-size of 350 was chosen because this was the recommended sample size for use of PFC for market-assurance and surveillance testing. A cut-off of 2% was also chosen because sample sizes for market-assurance testing were determined to provide 95% confidence of detecting a prevalence of 2%.

**Estimation of flock-sensitivity of both tests**

**Estimation of flock-sensitivity assuming both tests are 100% specific**

Staquet et al. (1981) described a method for estimating the sensitivities of two tests applied at the individual level, where both tests have 100% specificity. This method was adapted to estimate the flock-sensitivities for both PFC and serology (see Table 4.1 and equations 1 and 2), where $S_{e_{pfc}}$ and $S_{e_{ser}}$ were the estimated flock-sensitivities of PFC and serology/histology respectively (Staquet et al., 1981). Flock-sensitivities for both tests were estimated across all flocks in the study and for low (<2%) and high (≥2%) prevalence flocks separately.

**Table 4.1. Estimation of the flock-sensitivity of two tests, if both tests have 100% flock-specificity and if there is no gold standard.**

<table>
<thead>
<tr>
<th>PFC (Test 1)</th>
<th>Serology/histology (Test 2)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>+ve</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>-ve</td>
<td>c</td>
<td>d</td>
</tr>
<tr>
<td>Total</td>
<td>a+c</td>
<td>b+d</td>
</tr>
</tbody>
</table>

$S_{e_{pfc}} = a/(a+c)$ \hspace{5cm} (1)

$S_{e_{ser}} = a/(a+b)$ \hspace{5cm} (2)
A McNemar’s test was used to test the statistical significance of differences between flock-sensitivity estimates for the two tests, with a $P$-value <0.05 (two-tailed) regarded as significant (Thrusfield, 1995, p 214).

**Bayesian estimation of flock-sensitivity and specificity**

Bayesian analysis using a Gibbs sampler investigated the potential effects of imperfect flock-specificity of PFC, and evaluated the effect of incorporating prior knowledge of the performance of these tests on the resulting flock-sensitivity and flock-specificity estimates (Joseph et al., 1995; Enøe et al., 2000).

A Gibbs sampler based on that used by Joseph et al. (1995) was developed using WinBUGS software (Spiegelhalter et al., 1999), and was used to estimate posterior distributions for the flock-sensitivities and flock-specificities of the two tests and the prevalence of infected flocks in the sample (Joseph et al., 1995).

This method requires input of:

- $\alpha$ and $\beta$ parameters for five prior Beta distributions: for flock-prevalence, and for flock-sensitivity and flock-specificity of both tests;
- starting values for flock-prevalence and flock-sensitivity and flock-specificity of both tests; and
- starting values for the estimated number of truly infected animals in each cell of the two-by-two table describing the test comparison.

Parameters and starting values for the prior distributions for flock-sensitivities and flock-specificities and the prevalence of infected flocks were as follows: Flock-specificity for serology/histology was assumed to be 100%, because the flock-diagnosis
was based on confirmation of infection in individual seropositive animals using histology (which is 100% specific at the animal-level). A starting value of 100% and a strong prior distribution assuming 0/1000 false positives for serology/histology were used, with $\alpha = 1001$ and $\beta = 1$. The prior distribution and starting value for PFC flock-specificity were based on the estimated flock-specificity from the analysis of testing results. In this case, $\alpha = (\text{number of submissions} - \text{false positives} + 1)$, $\beta = (\text{false positives} + 1)$ (Vose, 2000, p 71), and the point estimate of flock-specificity was used as the starting value.

Uninformed (uniform) prior distributions were used for flock-sensitivities for both tests, because these were the parameters of primary interest, and there was considerable uncertainty about the actual flock-sensitivity that would be achieved considering the variation in estimated prevalence and sample size observed in the study flocks. The prevalence of infected flocks in the study was also unknown, so that an uninformed prior distribution and a starting value of 50% also were used for prevalence. These inputs were described as Beta distributions with parameters $\alpha = 1$ and $\beta = 1$ (which equates to a uniform distribution with a minimum value of 0 and maximum value of 1) and in each case a starting value of 0.5 was used.

Given the presumed high flock-specificity of both tests, the actual number of flocks observed in each cell of Table 1 were used as starting values for each of cells a, b and c. For cell d, the number of infected but undetected flocks was estimated from the starting value for flock prevalence, less the estimated number of flocks already identified in cells a, b and c.
The model was run for 25,000 iterations, with the results from the first 5,000 iterations discarded to allow convergence of the model and the results from the subsequent 20,000 iterations used to generate posterior distributions for the flock-sensitivities and flock-specificities of both tests and prevalence of infection. Posterior distributions were estimated using all flocks in the study, as well as in the high and low prevalence subgroups of flocks. The analysis was repeated using uninformed prior distributions for all parameters and the resulting output distributions were compared to those from the original model.

Because this method might be sensitive to changes in starting values and prior distributions, the model for all flocks was re-run with a range of different starting values and prior distributions. Alternative prior distributions were included assuming the flock-sensitivities of both tests were either 90% or 95% (because 95% was the target flock-sensitivity of the testing strategy), or assuming flock-specificity of PFC was 95% or 99%. Alternative starting values used were generally extreme values within the valid range for the parameter. The analysis also was repeated for 250,000 iterations instead of the original 25,000 to check for convergence and to ensure that the results were repeatable.

Effect of conditional dependence between test sensitivities

Both the above methods used for estimation of flock-sensitivities of the two tests assume that the two tests are independent, conditional on infection status. Briefly, if two tests are applied in an infected population, the covariance of the test flock-sensitivities
can be estimated as $\text{Cov}_{\text{Se}} = P_{++} - \text{Se}_{\text{pfc}} \cdot \text{Se}_{\text{ser}}$ where $\text{Se}_{\text{pfc}}$ and $\text{Se}_{\text{ser}}$ are the flock-sensitivities of the two tests and $P_{++}$ is the proportion of infected flocks that test positive to both tests. If $P_{++} = \text{Se}_{\text{pfc}} \cdot \text{Se}_{\text{ser}}$, then $\text{Cov}_{\text{Se}} = 0$ and the test flock-sensitivities are independent (Vacek, 1985; Gardner et al., 2000; Dendukuri and Joseph, 2001). If $\text{Cov}_{\text{Se}} > 0$, then the test flock-sensitivities are correlated positively.

Because the number of infected flocks that were not detected by either test was not known, it was not possible to determine true values for $\text{Se}_{\text{pfc}}$, $\text{Se}_{\text{ser}}$ and $\text{Cov}_{\text{Se}}$. However, using the test results for all flocks in the field trial, and assuming that both tests had a flock-specificity of 100%, values for $\text{Se}_{\text{pfc}}$, $\text{Se}_{\text{ser}}$ and $\text{Cov}_{\text{Se}}$ were estimated separately for assumed numbers of undetected infected flocks ranging between 0 and 50.

4.3 Results

4.3.1 Flock-specificity of pooled faecal culture

A total of 392 laboratory submissions from 31 districts were available for analysis, with 227 of these being from 25 districts with <20 known infected flocks. There were nine faecal culture-positive submissions from these districts, of which seven were from properties that subsequently were confirmed as infected. On both remaining culture-positive properties, a positive PCR result was obtained on growth in BACTEC — but the organism was not isolated on solid media (either on primary culture or on sub-culture from BACTEC), and infection was not confirmed by further investigation using serology and repeated PFC.
There were thus two apparent false positives from 227 eligible submissions, and the estimated minimum flock-specificity of pooled faecal culture in the field was 99.1 (96.9%, 99.9%). α and β parameters for the prior distribution in the Bayesian analysis were 226 and 3 respectively, and a starting value of 99.1% was used.

4.3.2 Prevalence in infected flocks

Of 296 flocks investigated during the field trial, 100 (34%) were identified as infected by one or both tests, with a further 12 flocks inconclusive on either PFC (9) or serology/histology (3). For all additional analyses, these inconclusive results were regarded as being test negative for that test. A total of 162 flocks had <350 sheep tested (72 flocks had ≤50 sheep) and 134 had ≥350. About 25% of the flocks with <350 sheep tested were positive to one or both tests compared to 44% of flocks that had ≥350 tested.

Sample sizes for serological testing in the positive flocks ranged from 40 to 500, (41 flocks had <350 sheep tested, including 19 flocks with ≤50 tested). Seroprevalence in these flocks ranged from 0 to 26%, with 24 flocks seronegative. The estimated true prevalence after adjusting for an assumed sensitivity of 30% for the AGID ranged from 0.5% to 87%. Overall, 46% of flocks had an estimated true prevalence <2%, and 14% of flocks had an estimated prevalence of >10%. Only 17% (7/41) of positive flocks with <350 sheep tested had an estimated prevalence of <2%, compared to 66% (39/59) of those with ≥350 tested. Eighteen of the 24 positive flocks that were seronegative had ≥350 sheep tested.
4.3.3 Flock-sensitivity of both tests

Estimation of flock-level sensitivity assuming both tests are 100% specific

Overall, the estimated flock-sensitivity of PFC in the study population was 92% (82%, 97%), compared to 61% (51%, 71%) for serology/histology (Table 4.2). Serology performed much worse in lower prevalence flocks. PFC also performed better than serology in higher prevalence flocks, but the difference was not as great. Differences in flock-sensitivity estimates for the two tests were statistically significant \((P < 0.001)\) except in high-prevalence flocks \((P = 0.11)\).

Table 4.2. Comparison of flock-sensitivities of PFC and serology in 296 sheep flocks tested in New South Wales during 1998 and in 46 low-prevalence and 54 high-prevalence flocks in the same study, assuming both tests were 100% specific.

<table>
<thead>
<tr>
<th>Population</th>
<th>PFC/Serology Test results</th>
<th>Sensitivities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+ (+)</td>
<td>Se\textsubscript{pfc} (%)</td>
</tr>
<tr>
<td>All flocks</td>
<td>58 (a)</td>
<td>92</td>
</tr>
<tr>
<td>Low-prevalence flocks</td>
<td>14 (b)</td>
<td>82</td>
</tr>
<tr>
<td>High-prevalence flocks</td>
<td>44 (c)</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>+/– (+)</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>–/+ (+)</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>–/– (+)</td>
<td>85</td>
</tr>
</tbody>
</table>

Bayesian estimation of flock-sensitivity and specificity

The posterior distributions from the Gibbs sampling model for data from all flocks (Table 4.3) indicated median flock-specificities of 98.8% (96.8%, 99.7%) for PFC, and 99.9% (99.6%, 100%) for serology/histology. Estimates of the flock-sensitivities (Tables 4.3 and 4.4) were comparable to estimates derived using the alternative method (Table 4.2). Use of uninformed prior distributions for all parameters resulted in a substantial reduction in flock-specificity estimates and a corresponding increase in flock-sensitivity estimates for both tests (Table 4.3).
Table 4.3. Posterior distributions for flock-sensitivities and flock-specificities of PFC and serology, from the Bayesian analysis of data from 296 sheep flocks tested in New South Wales during 1998.

<table>
<thead>
<tr>
<th>Simulation</th>
<th>Parameter</th>
<th>Mean (%)</th>
<th>Median (%)</th>
<th>95% PI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>Sp_{pfc}</td>
<td>98.7</td>
<td>98.8</td>
<td>96.8 – 99.7</td>
</tr>
<tr>
<td>2†</td>
<td>Sp_{pfc}</td>
<td>90.4</td>
<td>90.1</td>
<td>81.7 – 99.2</td>
</tr>
<tr>
<td>1</td>
<td>Sp_{ser}</td>
<td>99.9</td>
<td>99.9</td>
<td>99.6 – 100</td>
</tr>
<tr>
<td>2</td>
<td>Sp_{ser}</td>
<td>98.2</td>
<td>98.5</td>
<td>95.3 – 99.9</td>
</tr>
<tr>
<td>1</td>
<td>Se_{pfc}</td>
<td>91.0</td>
<td>91.4</td>
<td>82.9 – 96.8</td>
</tr>
<tr>
<td>2</td>
<td>Se_{pfc}</td>
<td>94.3</td>
<td>95.0</td>
<td>85.3 – 99.7</td>
</tr>
<tr>
<td>1</td>
<td>Se_{ser}</td>
<td>62.0</td>
<td>62.2</td>
<td>51.9 – 72.2</td>
</tr>
<tr>
<td>2</td>
<td>Se_{ser}</td>
<td>78.2</td>
<td>77.5</td>
<td>57 – 98.8</td>
</tr>
</tbody>
</table>

* Simulation 1 - Prior distributions assume high flock-specificity for both tests.
† Simulation 2 - Uniform prior distributions used for flock-specificity for both tests.

Variations in prior input distributions and starting values appeared to have little effect on the estimated flock-sensitivity of PFC: all estimates were within ±2% — despite some quite-extreme changes to input values (data not shown). In contrast, estimates of the flock-sensitivity of serology/histology were highly unstable, varying by up to 23%. Most of these variations were upwards and were associated with either a prior distribution assuming a high flock-sensitivity for serology, or a substantial reduction in the flock-specificity of PFC.

Under most scenarios tested, the median flock-specificity of both tests remained at or above 99% for PFC, and close to 99.9% for serology/histology. Even when the posterior-median flock-specificity of PFC was as low as 90%, its posterior-median flock-sensitivity remained high at 95%, while that for serology/histology increased to 78% (see Table 4.3).
Table 4.4. Comparison of Bayesian estimates of flock-sensitivities for PFC and serology with estimates assuming both tests were 100% specific in 296 sheep flocks tested in New South Wales during 1998.

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameter</th>
<th>Mean (%)</th>
<th>95% Interval (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All flocks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bayesian</td>
<td>Se_pfc</td>
<td>91.0</td>
<td>82.9 – 96.8</td>
</tr>
<tr>
<td>Both tests 100% specific</td>
<td>Se_pfc</td>
<td>92.1</td>
<td>82.4 – 97.4</td>
</tr>
<tr>
<td>Bayesian</td>
<td>Se_ser</td>
<td>62.0</td>
<td>51.9 – 72.2</td>
</tr>
<tr>
<td>Both tests 100% specific</td>
<td>Se_ser</td>
<td>61.1</td>
<td>50.5 – 70.9</td>
</tr>
<tr>
<td><strong>Low-Prevalence flocks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bayesian</td>
<td>Se_pfc</td>
<td>79.6</td>
<td>58.9 – 94.7</td>
</tr>
<tr>
<td>Both tests 100% specific</td>
<td>Se_pfc</td>
<td>82.4</td>
<td>56.6 – 96.2</td>
</tr>
<tr>
<td>Bayesian</td>
<td>Se_ser</td>
<td>34.4</td>
<td>20.8 – 50.1</td>
</tr>
<tr>
<td>Both tests 100% specific</td>
<td>Se_ser</td>
<td>32.6</td>
<td>19.1 – 48.5</td>
</tr>
<tr>
<td><strong>High-Prevalence flocks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bayesian</td>
<td>Se_pfc</td>
<td>94.0</td>
<td>85.6 – 99.1</td>
</tr>
<tr>
<td>Both tests 100% specific</td>
<td>Se_pfc</td>
<td>95.7</td>
<td>85.2 – 99.5</td>
</tr>
<tr>
<td>Bayesian</td>
<td>Se_ser</td>
<td>87.3</td>
<td>75.0 – 97.7</td>
</tr>
<tr>
<td>Both tests 100% specific</td>
<td>Se_ser</td>
<td>84.6</td>
<td>71.9 – 93.1</td>
</tr>
</tbody>
</table>

The model converged rapidly, and results were highly repeatable. Estimates derived from the results of 200,000 iterations after allowing for 50,000 iterations for convergence were no different from the original estimates. Similarly, median estimates for all parameters calculated from consecutive groups of 50,000 iterations were within 0.1% of each other and of the original estimates for all parameters, with similar 95% probability limits.

_Effect of conditional dependence between test sensitivities_

If there were three infected flocks that were not detected by either test then Cov_{Se} was zero and the flock-sensitivities of the two tests were independent. As the assumed number of undetected flocks increased, Cov_{Se} also increased and the true flock-sensitivities for both PFC and serology/histology decreased (flock-sensitivity of PFC

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dropped faster than that of serology/histology). Despite this, even if there were up to 50 undetected infected flocks, the estimated flock-sensitivity of PFC was still substantially higher than that for serology/histology (Table 4.5). Fewer than three additional infected flocks would result in a negative correlation between the tests, a result that is regarded as unlikely (Gardner et al., 2000).

Table 4.5. Effect of the assumed number of false-negative flocks on flock-sensitivities of PFC and serology, the covariance of test sensitivities and OJD prevalence estimates in 296 sheep flocks tested in New South Wales during 1998.

<table>
<thead>
<tr>
<th>Assumed number of infected flocks negative to both tests</th>
<th>$S_{\text{pfc}}$ (%)</th>
<th>$S_{\text{ser}}$ (%)</th>
<th>Covariance of test sensitivities</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>63</td>
<td>-0.02</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>92</td>
<td>61</td>
<td>0.00</td>
<td>35</td>
</tr>
<tr>
<td>10</td>
<td>86</td>
<td>57</td>
<td>0.03</td>
<td>37</td>
</tr>
<tr>
<td>20</td>
<td>79</td>
<td>53</td>
<td>0.07</td>
<td>41</td>
</tr>
<tr>
<td>30</td>
<td>73</td>
<td>48</td>
<td>0.09</td>
<td>44</td>
</tr>
<tr>
<td>40</td>
<td>68</td>
<td>45</td>
<td>0.11</td>
<td>47</td>
</tr>
<tr>
<td>50</td>
<td>63</td>
<td>42</td>
<td>0.12</td>
<td>51</td>
</tr>
</tbody>
</table>

4.4 Discussion

Both tests evaluated in this study are biologically 100% specific at a flock level. Serological screening relies on the observation of typical lesions containing acid-fast bacilli in tissue samples from serological reactors to confirm infection, whereas PFC depends on identification of $M$ a paratuberculosis by PCR for a sequence assumed to be unique to $M$ a paratuberculosis or by isolation and identification of the organism on solid medium. Therefore, false-positive results for PFC only should occur through contamination or mis-identification of samples in either the field or laboratory. Strict quality-control measures were used to ensure that contamination did not occur in the laboratory, and guidelines for field collection and submission of samples were provided to minimise the chance of any cross-contamination between samples from different
farms. In this context, cross-contamination of samples is most likely to occur between pools for an infected flock, with no effect on the flock-specificity of the test. Cross-contamination between flocks is less likely — but would result in a reduction in flock-specificity.

The flock-specificity of PFC was estimated using the results of general surveillance testing after removal of known infected animals or flocks. In this case, the true flock-specificity of the test generally will be underestimated — but the estimate will be close to the true level (particularly if disease prevalence is low and known-infected animals or flocks can be identified reliably and excluded from the data) (Seiler, 1979). This analysis supported the assumed high flock-specificity of PFC, with only two apparent false-positive results out of 227 submissions tested. This provides a minimum estimate of flock-specificity, because one or both of these flocks might have been infected (but at an early stage or very low prevalence). Both of these submissions yielded only PCR positives and could not be cultured on solid media — suggesting that the level of growth was low (supporting the interpretation of minimum flock-specificity).

The use of biased sampling in some flocks might have over-estimated true prevalence in those flocks. Similarly, the method used to estimate true prevalence in seronegative flocks may have over- or under-estimated the true prevalence in those flocks. However, the intention was to categorise flocks simply as high or low prevalence, and these biases are unlikely to have affected the comparison greatly between sub-populations in this study.
The estimated flock-sensitivity of pooled faecal culture suggests that it is performing at an acceptable level, and is likely to be achieving close to the desired level of confidence when used for surveillance and assurance testing. The guidelines for the market-assurance program require testing a sample size of 350 sheep using PFC in seven pools of 50 each, or up to 500 sheep using serology, to provide 95% confidence of detecting a prevalence of 2% (equivalent to a flock-sensitivity of 95% for flocks with 2% prevalence). In this study, a flock-sensitivity for PFC of 96% (85%, 99.5%) was achieved in flocks with an estimated prevalence ≥2%, and 82% (57%, 96%) in flocks with a lower prevalence. In contrast, serology/histology failed to achieve the desired level of confidence (even in flocks with higher prevalence). A sample size much larger than currently recommended might be required to provide satisfactory flock-sensitivity for serology in low-prevalence flocks. Alternatively, we might have to lower flock-sensitivity to an unacceptable level.

Generally, faecal culture is a more-sensitive animal-level test than serology, and faecal shedding begins before humoral responses in most animals (Chaitaweesub et al., 1999; Whittington and Sergeant, 2001). Thus, complete independence of tests is unlikely, and the sensitivities of serology and PFC are likely to be correlated in infected animals and flocks. More-precise estimates of flock-sensitivity adjusted for any correlation between tests were not possible in this study without knowing the true status of all flocks in the study. Thus it is impossible to know the degree of over-estimation that may have occurred and the estimates provided here must be regarded as maximum values rather than true unbiased estimates.
The flocks included in this study were tested as part of ongoing surveillance and market-assurance programs, and therefore are not necessarily representative of the general sheep population in Australia. In addition, flock-sensitivity is affected by within-flock prevalence of disease, and thus varies between flocks (Martin et al., 1992). Thus, the estimates of flock-sensitivity presented here represent a weighted average for the flocks included in the study and actual sensitivity in individual flocks will vary depending on the characteristics of the flock (especially with respect to the stage of disease in infected sheep).

4.5 Conclusion

These results confirm that PFC is a highly sensitive and specific flock-test for detection of ovine Johne’s disease — and is substantially more sensitive than serology. The difference in performance between the two testing approaches was particularly pronounced in apparently low-prevalence flocks. In such flocks, serology performed extremely poorly. If the flock-sensitivities of the two tests are correlated then both PFC and serology are both likely to have a lower flock-sensitivity than these estimates.

The current sample size of 350 sheep per flock for surveillance and market-assurance testing using PFC should provide the required 95% flock-sensitivity in flocks with a prevalence of 2% or greater, and a reduced but still-acceptable flock-sensitivity in lower-prevalence flocks. In contrast, the sample sizes used for serology in this study don’t provide 95% flock-sensitivity — even in higher-prevalence flocks.
Part 3

Prevalence and distribution of OJD in Australia and risk assessment
5. The estimated prevalence of Johne’s disease infected sheep flocks in Australia

5.1 Introduction

A key objective of the NOJDP is to determine the distribution and prevalence of OJD among flocks in Australia (see Chapter 2). However, because of the insidious nature of the disease, and the large proportion of infected animals that are sub-clinical carriers, prevalence of OJD is very difficult to estimate reliably. Up until 1999, surveillance for OJD mainly concentrated on the investigation of suspect clinical cases or of flocks identified as being at higher risk through contact with known infected flocks. As a result, properties in known infected areas, or those that have purchased sheep from these areas have been preferentially selected for surveillance. Since mid-1997, market-assurance testing has provided additional surveillance data, including some data for areas where little testing had been done previously (Chapter 2). Thus, any estimates of flock-prevalence based on existing surveillance data are likely to be significantly biased, with the direction and magnitude of the bias likely to vary considerably between regions, depending on the trading patterns and infection history of the region.

A large-scale survey to estimate the flock-prevalence of OJD by on-farm testing using serology or other tests was not considered feasible, because of the very high cost and the potential for producer resistance to testing to significantly bias any results. Therefore, an alternative method was required that was not affected by the selective nature of surveillance testing or producer resistance to testing.
From late 1999, abattoir surveillance was introduced in most Australian States as an alternative method of surveillance for OJD (Chapter 2). Although abattoir surveillance is not biased towards perceived high-risk flocks in the same way as other methods, it is still subject to a range of biases, and is also affected by an unknown and variable sensitivity as a flock-screening test. Therefore, although it is possible to estimate flock-prevalence from abattoir surveillance data, any such estimates must still be treated with some caution.

Estimation of the prevalence of OJD is further complicated by the imperfect sensitivity of abattoir surveillance, and any prevalence estimates are therefore likely to substantially underestimate the true prevalence of the disease unless adjusted for the sensitivity and specificity of the test used. Adjustment of prevalence estimates is also complicated by the fact that the flock-sensitivity of abattoir surveillance is not known, and may vary between flocks and regions, introducing further uncertainty into any prevalence estimates.

Mathematical models can be used to solve real-world problems such as this by translating them into mathematical descriptions that can then be analysed and solved using standard mathematical techniques (Murthy et al., 1990). Therefore, although the true flock-sensitivity of abattoir surveillance is unknown, a simple mathematical model can be used to estimate the flock-sensitivity under a given set of assumptions about prevalence, number of sheep examined and percentage with detectable gross lesions.

Similarly, mathematical simulation using a Gibbs sampler can be used to solve the
complex mathematical relationships required to adjust apparent flock-prevalence for the assumed flock-sensitivity and flock-specificity of abattoir surveillance and our uncertainty about these values (Joseph et al., 1995; Enøe et al., 2000).

The objective of this study was to estimate the flock-prevalence and geographical distribution of OJD in Australia, using abattoir surveillance data adjusted for the imperfect sensitivity and specificity of the test.

5.2 Materials and Methods

The prevalence of OJD-infected flocks was estimated separately for three geographic regions of New South Wales, and for each of the States of Queensland, South Australia, Tasmania, Victoria and Western Australia. The Northern Territory has no commercial sheep flocks and the Australian Capital Territory has only 85 flocks and was not included in this analysis. Flock-prevalence for each region of New South Wales was estimated and compared using three different methods: by extrapolation from the number of known infected flocks, from the results of laboratory investigations and from the results of abattoir surveillance. For States other than New South Wales, flock-prevalence was estimated from the results of abattoir surveillance only.

Because the prevalence of known OJD-infected flocks varied markedly across New South Wales, Rural Lands Protection Board districts in that State were allocated to one of three regions based on their prevalence of known infected flocks at 31 December 2000. Districts with <0.5% known infected flocks were allocated to low prevalence (LP) regions, districts with 0.5% to 5% were moderate prevalence (MP) and districts with >5% known infected flocks were high prevalence (HP) regions. For the other
prevalence was estimated for the whole State, except that data for Kangaroo Island in South Australia and Flinders Island in Tasmania were excluded from the analysis. These islands have a relatively small number of sheep flocks but a high flock-prevalence of OJD compared to the rest of the State, and were thus likely to bias any overall prevalence estimates, if included. There was insufficient data to allow estimation of prevalence for these islands separately.

5.2.1 Estimated flock-prevalence from known infected flocks

The prevalence of infected flocks in each region of New South Wales was first estimated from data on known infected flocks. For each region, the total number of flocks, the number of known infected flocks at 31 December 2000 and the number of additional properties identified as neighbours or as having purchased sheep from known infected properties were calculated from OJD surveillance data provided by the Rural Lands Protection Boards.

Based on analysis of New South Wales’ surveillance data, it was assumed that about 30% of traced properties were infected across all regions and that the percentage of neighbours that were infected increased from 20% in the LP region to 40% and 50% in the MP and HP regions respectively. For each region, the numbers of neighbouring and traced properties that were assumed to be infected were calculated by multiplying the total number of neighbouring or traced properties by the corresponding percentages that were assumed to be infected. The prevalence of infected flocks in each region was then estimated as the number of current known infected flocks, plus the estimated numbers of traced and neighbouring flocks assumed to be infected, divided by the total number of flocks for the region.
Estimated flock-prevalence from the results of laboratory investigations

The results of all laboratory testing for OJD undertaken in New South Wales between 1 April 1998 and 31 December 2000 were summarised by property of origin and the flock-prevalence for each region was estimated as the percentage of properties investigated that had a positive result on either histology or pooled faecal culture.

Estimated flock-prevalence from abattoir surveillance data

Abattoir surveillance commenced in New South Wales in late 1999 as a screening procedure for the detection of OJD-infected flocks. Trained staff, usually former meat inspectors, routinely examined lines of sheep at a number of abattoirs throughout New South Wales, where a line of sheep was a number of sheep killed as a group, so that individuals within the line came from the same property or properties of origin. Individual sheep were examined manually and visually for the presence of any lesions in the intestinal tract or associated lymph nodes that were suggestive of OJD. Tissue samples were collected for histological examination from up to three sheep with lesions in each line of sheep in which lesions were detected. A line of sheep was positive on abattoir surveillance if one or more sheep from the line had histological lesions consistent with paratuberculosis (Cousins et al., 2001). A line of sheep was negative on abattoir surveillance if either there were no gross lesions detected or all gross lesions sampled from the line were negative for OJD on histological examination.

The prevalence of infected flocks in each region was estimated from abattoir surveillance data, after adjusting for the assumed sensitivity and specificity of abattoir
surveillance. Briefly, a Bayesian approach was used which allows combination of any prior information available on test sensitivity and specificity and flock-prevalence of disease with the results of abattoir surveillance, to produce a probability distribution of the estimated flock-prevalence (Joseph et al., 1995). A Gibbs sampler based on that used by Joseph et al. (1995) for estimation of prevalence using a single test was developed with WinBUGS software (Spiegelhalter et al., 1999), and was used to estimate the prevalence of infected flocks in each region.

This method requires input of prior estimates for the average flock-sensitivity, average flock-specificity and flock-prevalence as Beta probability distributions, as well as initial assumed values for flock-sensitivity, flock-specificity and flock-prevalence. The prior estimates of flock-sensitivity, flock-specificity and flock-prevalence are estimates of the most likely values of these parameters, based on previous studies, alternative sources of data or expert opinion. Beta distributions were used because these distributions are commonly used to describe uncertainty about the true value of a proportion, such as sensitivity or prevalence, and are appropriate distributions for use in the Gibbs sampler (Joseph et al., 1995; Vose, 2000). When used for this purpose, the Beta distribution can be defined by the two parameters, $\alpha$ and $\beta$, with $\alpha = x + 1$ and $\beta = n - x + 1$, where $x$ is the number of positive events out of $n$ trials. As $n$ increases, the degree of uncertainty (the width of the distribution) about the estimated proportion ($x/n$) decreases.

Prior distributions and initial values for flock-sensitivity and flock-specificity of abattoir surveillance and flock-prevalence of infection were derived as follows:
Prior distribution for the flock-sensitivity of abattoir surveillance

The flock-sensitivity of abattoir surveillance for OJD is unknown, and is likely to vary depending on the prevalence of infection in infected flocks, the proportion of animals with gross lesions (animal-level sensitivity), the number of animals examined, and the efficiency of the inspector (the proportion of gross lesions detected). Therefore, an @Risk (Palisade Corporation) simulation model was used to estimate the flock-sensitivity of abattoir surveillance for the LP, MP and HP regions of New South Wales separately. The model simulated 1,000 infected flocks and was run for 1,000 iterations for each region to produce a probability distribution for the estimated flock-sensitivity of abattoir surveillance for the region.

Model parameters were input as probability distributions that were varied between the scenarios to reflect the expected variations between regions. Input parameters for the model and their values for each of the three regions of NSW are listed in Table 5.1. The number of animals examined per line of sheep was determined by selecting at random from a general distribution based on the actual number of sheep examined per line in NSW during the period November 1999 to 31 March 2000. Pert distributions, which were used for all other input distributions, are smoothed triangular distributions that can be easily defined using minimum, most likely and maximum values and are commonly used to model expert opinion about the value of a variable (Vose, 2000).

For each simulated flock, the model determined the number of sheep examined, the number of these that were infected, the number with gross lesions, the number with gross lesions that were detected (depending on the efficiency of the inspector), the
The number of sheep inspected with false positive lesions (gross lesions suggestive of OJD but due to something else) and the number of sheep with lesions that were detected, as shown in Figure 5.1. The flock-sensitivity for each iteration was calculated as the proportion of simulated flocks in which one or more infected animals were detected.

Table 5.1. Input parameters and their values for a simulation model to estimate the flock-sensitivity of abattoir surveillance in three regions of New South Wales

<table>
<thead>
<tr>
<th>Input parameter</th>
<th>Regiona</th>
<th>Distribution</th>
<th>Minimum value</th>
<th>Most likely value</th>
<th>Maximum value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sheep examined</td>
<td>All</td>
<td>General</td>
<td>1</td>
<td></td>
<td>1000</td>
</tr>
<tr>
<td>Prevalence in flock (PREV)</td>
<td>LP</td>
<td>Pert</td>
<td>1%</td>
<td>2%</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>MP</td>
<td>Pert</td>
<td>1%</td>
<td>5%</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>HP</td>
<td>Pert</td>
<td>2%</td>
<td>10%</td>
<td>40%</td>
</tr>
<tr>
<td>Percentage of animals with gross lesions (SENS)</td>
<td>LP</td>
<td>Pert</td>
<td>5%</td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>MP</td>
<td>Pert</td>
<td>5%</td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>HP</td>
<td>Pert</td>
<td>5%</td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td>Efficiency of inspector (INSPECTOR)</td>
<td>All</td>
<td>Pert</td>
<td>80%</td>
<td>90%</td>
<td>99%</td>
</tr>
<tr>
<td>Percentage of uninfected animals in false positive lines with lesions (FPPC1)</td>
<td>All</td>
<td>Pert</td>
<td>1%</td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td>Background percentage of uninfected animals with lesions (FPPC2)</td>
<td>All</td>
<td>Pert</td>
<td>0.05%</td>
<td>0.10%</td>
<td>0.30%</td>
</tr>
<tr>
<td>Percentage of lines with higher % false positives (FPLINES)</td>
<td>All</td>
<td>Fixed</td>
<td>2.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum number sampled (MAXSAMPLES)</td>
<td>All</td>
<td>Fixed</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Regions of NSW - LP: low prevalence; MP: moderate prevalence; HP: high prevalence

Minimum, most likely and maximum values for within-flock prevalence were based on the assumption that within-flock prevalence is likely to be lower in lower prevalence regions, as the disease has not been present as long within these flocks. Input values for prevalence for the LP and MP regions were consistent with estimates from laboratory testing data (details not shown), while values for the HP region were consistent with estimates of histological prevalence in six flocks from this area (Marshall et al., 1996; Marshall et al., 1997). The percentage of animals with detectable lesions was also

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assumed to be higher in the HP region than in the LP or MP regions. Based on the results of abattoir surveillance undertaken in New South Wales, false positive lesions were assumed to occur at very low rates in the general sheep population, but at higher levels in 2.5% of lines (see Table 5.1).

Figure 5.1. Diagram of the scenario tree and @Risk functions used to determine whether an OJD-infected flock was detected by simulated abattoir surveillance for OJD
The median of the @Risk output distribution, rounded to the nearest 5%, was used as the starting value for the Gibbs sampler for each region. The $\alpha$ and $\beta$ parameters for the prior distributions were determined to provide diffuse (wide) distributions around the starting value.

**Prior distribution for the flock-specificity of abattoir surveillance**

Flock-specificity for abattoir surveillance was assumed to be 100% for all regions, because it is based on the identification of characteristic histologic lesions containing acid-fast bacilli in animals with suspect lesions. A starting value of 100% and a prior distribution assuming 0/200 false positives were used, with $\alpha = 201$ and $\beta = 1$.

**Prior distribution for the flock-prevalence of OJD**

Prior estimates of prevalence for each region were based on extrapolation from the number of known infected flocks as described above. $\alpha$ and $\beta$ parameters for the prior distributions for prevalence were estimated to provide probability distributions about the point estimates that were equivalent to an assumed sample size of 100 lines, to provide 95% confidence limits of about $\pm 10\%$ around the estimates. The point estimate of prevalence was used as the starting value for the distribution for each region.

**Apparent flock-prevalence from abattoir surveillance**

The Gibbs sampler also requires input of the number of lines of sheep that were positive on abattoir surveillance and the number of lines that were negative. For each region, the numbers of positive and negative lines were determined from abattoir surveillance results for the 2000 calendar year. Only lines of sheep that could be identified as originating from a specific Rural Lands Protection Board district within New South
Wales were included in the analysis. The apparent flock-prevalence of OJD was calculated as the percentage of lines examined that were positive for OJD.

**Estimated flock-prevalence using the Gibbs sampler**

The Gibbs sampler combines the apparent prevalence estimated from the abattoir surveillance data with sampled values from the prior distributions to generate a series of flock-prevalence estimates. Over a large number of repetitions the estimates generated converge on the flock-prevalence value that best fits the combination of prior distributions and the data. A posterior probability distribution for this value can then be generated from additional repetitions (Suess EA et al., 2000). For this analysis, the Gibbs sampler was run for 25,000 iterations for each region, with the results from the first 5,000 iterations discarded to allow convergence, and the results from the subsequent 20,000 iterations used to generate the probability distribution for the prevalence of infected flocks.

For States other than New South Wales, prevalence was estimated using the Gibbs sampler and the results of abattoir surveillance for the 12 months to December 2000 (Allworth, 2001; Anonymous, 2001a), except as follows. For South Australia, data to March 2001 was used, with data for Kangaroo Island excluded, and abattoir surveillance included the collection of faecal samples for pooled faecal culture, as well as manual and visual inspection. For Tasmania, lines from Flinders Island were excluded from the data. The same prior distributions for sensitivity and specificity of abattoir surveillance were used as for the LP region of New South Wales. Because all of these States were thought to have a relatively low prevalence of infected flocks, based on the prevalence of known infected flocks at 31 December 2000 (Allworth, 2001), a prior distribution
assuming 1/500 infected flocks was used for prevalence for these States, with $\alpha = 2$ and $\beta = 500$.

5.3 Results

Prevalence of known infected flocks by district in New South Wales ranged from 0% (29 districts) to 10%. The HP region comprised two districts in the central and southern tablelands area of the State, and the MP region included a further 10 districts in the central and southern parts of the State. The LP region comprised the remaining 36 districts from the northern, western and eastern parts of the State. The regional prevalence of known infected flocks was 0.07%, 1.6% and 9% for LP, MP and HP regions respectively, and the overall prevalence across New South Wales was 1.6%.

5.3.1 Estimated prevalence by extrapolation from known infected flocks

Based on analysis of surveillance and tracing data, the estimated flock-prevalence on a regional basis ranged from 0.2% in the LP region, to 7% and 30% in the MP and HP regions respectively.

5.3.2 Estimated prevalence from the results of laboratory investigations

A total of 1,868 property investigations were included in the analysis of laboratory data, with about 60% of these being in the MP region, and about 20% each in the HP and LP regions of New South Wales. Overall, 38% of investigations on properties in the HP region were positive, compared to 14% and 1.8% in the MP and LP regions respectively. Across all regions, 16% of properties investigated were positive on either histology or PFC or both.
5.3.3 Estimated flock-prevalence from abattoir surveillance data

The median estimated flock-sensitivity of abattoir surveillance for the LP region was 36%, compared to 57% and 89% for the MP and HP regions respectively. $\alpha$ and $\beta$ parameters for the prior distributions for abattoir surveillance sensitivity were 8 and 14, 12 and 10, and 19 and 3 for the LP, MP and HP regions respectively. These distributions had 95% probability limits of 18 and 57%, 34 and 74% and 70 and 97% respectively.

Table 5.2 summarises the results of abattoir surveillance by region and State. For New South Wales, 0.3% of lines from the LP region were positive, compared to 7 and 30% of lines from MP and HP regions respectively. All other States had 0.5% or less of lines positive.

Table 5.2. The number of lines of sheep examined at abattoirs for lesions due to OJD and the number and percentage of lines confirmed with OJD, for three regions of New South Wales and for other States of Australia from late 1999 to 31 December 2000.

<table>
<thead>
<tr>
<th>State/Region</th>
<th>Number examined</th>
<th>Number with OJD</th>
<th>Percentage with OJD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSW/LP</td>
<td>4383</td>
<td>15</td>
<td>0.3</td>
</tr>
<tr>
<td>NSW/MP</td>
<td>3927</td>
<td>281</td>
<td>7.2</td>
</tr>
<tr>
<td>NSW/HP</td>
<td>1388</td>
<td>417</td>
<td>30</td>
</tr>
<tr>
<td>Queensland</td>
<td>833</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>South</td>
<td>2851</td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
<td>Australia$^a$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tasmania$^b$</td>
<td>717</td>
<td>3</td>
<td>0.4</td>
</tr>
<tr>
<td>Victoria</td>
<td>3003</td>
<td>16</td>
<td>0.5</td>
</tr>
<tr>
<td>Western Australia</td>
<td>512</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ South Australian data were collected from late 1999 to March 2001, and do not include lines from Kangaroo Island

$^b$ Tasmanian data do not include lines from Flinders Island
The posterior distributions for OJD flock-prevalence, and the derived number of infected flocks, for each State and region are summarised in Table 5.3 and the comparison of results from the three different estimation methods used in New South Wales are shown in Table 5.4. Based on these estimates, about 6 to 10% of flocks in New South Wales and 2.4 to 4.4% of flocks Australia-wide were likely to be infected with OJD at 31 December 2000.

Table 5.3. The median and 95% probability limits of the posterior distributions for flock-prevalence of OJD, estimated using Gibbs sampler simulation, and the estimated number of infected flocks for three regions of New South Wales and for other States in Australia at 31 December 2000

<table>
<thead>
<tr>
<th>State*</th>
<th>Region/ Zone</th>
<th>Total flocks</th>
<th>Estimated prevalence (%)</th>
<th>Number of infected flocks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Median 95% probability limits</td>
<td>Median 95% probability limits</td>
</tr>
<tr>
<td>NSW</td>
<td>LP</td>
<td>16,783</td>
<td>0.5 0.05 – 1.5</td>
<td>90 8 – 256</td>
</tr>
<tr>
<td></td>
<td>MP</td>
<td>11,704</td>
<td>11 8 – 15</td>
<td>1,248 886 – 1,731</td>
</tr>
<tr>
<td></td>
<td>HP</td>
<td>3,388</td>
<td>33 29 – 39</td>
<td>1,124 994 – 1,310</td>
</tr>
<tr>
<td>NSW</td>
<td>All</td>
<td>31,875</td>
<td>7.9 6.3 – 10</td>
<td>2,518 2,008 – 3,188</td>
</tr>
<tr>
<td>Qld</td>
<td>CZ</td>
<td>3,215</td>
<td>0.2 0.03 – 0.5</td>
<td>7 1 – 24</td>
</tr>
<tr>
<td>SA**</td>
<td>CZ</td>
<td>8,500</td>
<td>0.2 0.03 – 0.5</td>
<td>14 2 – 43</td>
</tr>
<tr>
<td>Tas*</td>
<td>CZ</td>
<td>1,965</td>
<td>0.4 0.06 – 1.1</td>
<td>7 1 – 22</td>
</tr>
<tr>
<td>Vic</td>
<td>CZ</td>
<td>30,000</td>
<td>0.4 0.05 – 1.1</td>
<td>109 16 – 328</td>
</tr>
<tr>
<td>WA</td>
<td>FZ</td>
<td>8,727</td>
<td>0.2 0.04 – 0.8</td>
<td>21 3 – 73</td>
</tr>
<tr>
<td>Australia</td>
<td></td>
<td>84,282</td>
<td>3.2 2.4 – 4.4</td>
<td>2,677 2,032 – 3,678</td>
</tr>
</tbody>
</table>

* The 85 flocks in the ACT and 85 flocks in the residual zone of Tasmania were omitted because of the small numbers involved making estimations unreliable.
** SA estimates exclude Kangaroo Island.

5.4 Discussion

This study suggests that OJD has a highly clustered geographic distribution in Australia, and provides the first realistic estimates of the prevalence of infected flocks. Based on this analysis there were probably between 2,000 and 3,700 infected flocks in Australia at 31 December 2000, with more than 80% of these in a relatively small geographic area.
of central and southern New South Wales. Significant foci of infection also exist on Kangaroo Island in South Australia and Flinders Island in Tasmania, with other areas of Australia all having a likely flock-prevalence of about 1% or less. At 31 December 2000 there were 543 known infected flocks in Australia (Sergeant, 2001), so that only about 15 to 27% of all potentially infected flocks had been identified.

<table>
<thead>
<tr>
<th>Region</th>
<th>Extrapolated from known infected flocks (%)</th>
<th>From laboratory investigations (%)</th>
<th>Positive lines from abattoir surveillance (%)</th>
<th>Median estimated prevalence from abattoir surveillance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>0.2</td>
<td>1.8</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>MP</td>
<td>7</td>
<td>14</td>
<td>7.2</td>
<td>11</td>
</tr>
<tr>
<td>HP</td>
<td>30</td>
<td>38</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>All</td>
<td>6</td>
<td>16</td>
<td>7.4</td>
<td>7.9</td>
</tr>
</tbody>
</table>

Because of the nature of the available data, and uncertainty about the sensitivity of flock-screening tests for OJD, a novel approach was used to estimate flock-prevalence. This approach was subject to biases arising from the lack of representativeness of abattoir data and from any errors in the prior estimates of sensitivity, specificity and prevalence used. However, despite these shortcomings it was felt that a Bayesian approach made best use of the available data and provided adjusted estimates that included appropriate allowance for our uncertainty about the true values of the sensitivity and specificity of abattoir surveillance (Enøe et al., 2000).

A critical input for the Gibbs sampler was a prior estimate of the average sensitivity of abattoir surveillance as a flock-screening test. The flock-sensitivity of abattoir
surveillance is not well characterised and is likely to vary between flocks and between regions depending on the within-flock prevalence, the distribution of the disease in the flock, the representativeness of the line as a sample of the flock and the number of animals sampled. For example, in flocks where the only infected sheep have been recently purchased, these sheep are unlikely to be sold for slaughter, and the flock-sensitivity of abattoir surveillance will be zero. Conversely, in flocks with long-term infection at a high prevalence the flock-sensitivity of abattoir surveillance is likely to be high. Because of this variability, a simulation model was used to estimate the average flock-sensitivity of abattoir surveillance for LP, MP and HP regions. The simulated estimates were based on biologically realistic scenarios and data from the use of abattoir surveillance in Australia, and appeared reasonable for the different regions considered. In addition, diffuse distributions were used for prior estimates of flock-sensitivity in the Gibbs sampler, to reflect the high degree of uncertainty about these values.

The other key input that was required for the Gibbs sampler was a prior estimate of the flock-prevalence of OJD. For New South Wales, this was based on extrapolation from existing data to provide an approximate prevalence for each region, with a distribution allowing for a moderate degree of uncertainty about this value. For other States, a distribution assuming a prevalence of 1 in 500 lines was used. These distributions provide a starting point for the simulation process of the Gibbs sampler, and hence can influence the outcome if they are inaccurate or provide a higher than justified degree of certainty (Joseph et al., 1995). In this instance, the initial point estimates were at least consistent with alternative estimates from the available data and the distributions used allowed for a degree of uncertainty that was appropriate to each region/State. The
alternative of using an uninformed (uniform) prior distribution for the prior prevalence estimate would have failed to adequately take into account what we already knew about the prevalence and distribution of the disease. This would have resulted in much wider probability intervals and generally an over-estimation of the prevalence, particularly in the lower prevalence areas (Joseph et al., 1995; Enøe et al., 2000).

The final major source of potential bias in this analysis arises from the sampling method used for abattoir surveillance. Because abattoir surveillance relies on opportunistic sampling of sheep presented at abattoirs, the data may be biased by the fact that not all lines have been confirmed to their district or property of origin and many properties may have had multiple lines examined. In addition, the sheep examined at the abattoirs are not representative of the general sheep population. Despite these limitations, abattoir surveillance provides a large volume of data on flock testing that is not directed specifically at flocks likely to have the disease, and therefore provides the best currently-available opportunity for generating reasonable estimates of flock-prevalence of OJD.

Given the various potential sources of bias referred to above, the prevalence estimates presented here must be treated with some caution. However, comparison of these estimates with those generated by alternative approaches confirms that they are realistic and reasonable. For New South Wales, the median posterior prevalence estimates were slightly higher than the prior estimates for all regions. They were also lower than estimates based on the results of laboratory investigations, which would be expected to overestimate the prevalence due to the selection of high-risk flocks for investigation. In
other States, the results are generally consistent with the low assumed prevalence in these areas, although in some cases the upper probability limits are higher than desired due to the relatively small amount of abattoir surveillance undertaken.

From this analysis, the upper 97.5% probability limits for prevalence in Queensland and Western Australia were 0.7 and 0.8% respectively (upper 95% limits were 0.6 and 0.7% respectively). At the time of this study, OJD had never been detected in Queensland, and Western Australia was classified as a Free Zone. Therefore, although these estimates consider any pre-existing data in the prior prevalence estimate, they may still considerably over-estimate the true flock-prevalence in these States. This is particularly the case considering the relatively low numbers of lines examined, with only 833 examined in Queensland and 512 in Western Australia. A substantial amount of additional surveillance (many thousands of lines by abattoir surveillance) would be required to reduce the upper probability limits and confirm the very low prevalence assumed for these States.

Although the results presented here should be regarded as indicative rather than definitive, they suggest that OJD has a highly clustered geographic distribution in Australia and that many areas still have a very low prevalence, if the disease is present at all. Continuing surveillance and further revision and confirmation of these estimates as more data becomes available are essential for the ongoing management of OJD in Australia.
6. Risk-based trading options for ovine Johne’s disease

6.1 Introduction

Up until 2003, measures to limit the spread of OJD in Australia were based on quarantine of known or suspected infected flocks, restrictions on the movement of sheep between zones of differing prevalence and a market-assurance program (SheepMAP) to provide a source of low-risk sheep and to provide a basis for movement of sheep from higher to lower prevalence zones (See Chapter 2). This approach has a number of distinct shortcomings, including:

- The specific rules governing trade between zones, and between flocks with different SheepMAP status, were based largely on qualitative judgments of risk, which can be inconsistent;

- The imposition of quarantine on infected or suspect flocks and trading restrictions between zones has resulted in significant disruption to trade and severe financial and social consequences for affected producers, resulting in considerable producer opposition to the program;

- There is no provision for protection of neighbours of infected flocks, or for producers purchasing sheep from flocks of unknown infection-status within the same zone; and

- There is no recognition of the lower risk associated with producers taking positive steps to reduce the infection or to prevent its introduction (for example vaccination, grazing management, biosecurity measures, etc).
Therefore, an alternative risk-based approach is required that is scientifically-based to provide credibility, but at the same time is relatively simple and easily implemented by producers and others involved in the sheep industry. Implementation of a risk-based approach to OJD management could be entirely market-based, with voluntary (or compulsory) vendor declarations, and sanctions based only on common law provisions related to inadequate disclosure. Alternatively, the system could be supported by regulations directed at regional containment or eradication of the disease.

Importantly, such a system should:

- Allow a range of risk or mitigating factors to be taken into account when assessing the OJD-risk of a flock;
- Offer a range of risk-categories, so that producers can take small steps and yet make demonstrable progress towards expanding their trading options;
- Provide producers with an objective estimate of the risk of purchasing sheep from a flock with a given profile, relative to the risk associated with their own flock; and
- Provide incentives for producers to determine the infection-status of their flock or to implement control measures, including vaccination.

The objective of this study was to develop a simple and flexible method for estimating the flock-risk of OJD to support the possible future development of a risk-based approach to management of OJD in Australia.
6.2 Methods

6.2.1 Risk of OJD infection associated with individual flocks

Johne’s disease is transmitted primarily through contact of susceptible animals with infected faeces, and spread between properties may occur through either the planned movement of sheep between properties (sale/purchase or agistment), or from local spread via movement of sheep or faeces between adjoining properties or over commonly used ground (see Chapter 1).

Therefore, the risk of infectivity of a flock can be considered in two separate contexts:

1. The risk that it poses to neighbouring flocks and other flocks with which it has indirect contact, such as through common use of roads or facilities or environmental spread of infection; and

2. The risk that it poses to other, more distant flocks which purchase sheep from the infected flock or which have other direct contact such as through agistment of sheep.

For this project, ‘risk’ was defined as a quantitative index of the likelihood of an individual or group of sheep being infected. The likelihood of infection establishing on another property, the volume of trade between properties or regions, and the potential consequences if transmission and establishment of infection occurs were not considered in the risk assessment process.
The actual risk posed by an individual flock depends on whether or not the flock is infected and the level of infection if it is infected, and can be calculated as the product of the probability that the flock is infected and the probability that an individual within an infected flock is infected. These probabilities can be estimated as the flock-prevalence (proportion of flocks that are infected) among flocks with similar characteristics and the within-flock prevalence (proportion of animals that are infected) for infected flocks with similar characteristics, respectively. This flock-risk may then be modified by measures taken to reduce these probabilities (e.g. biosecurity, vaccination) and to reduce the risk of spread to other flocks (e.g. barriers to local spread, movement controls) or by factors which increase these probabilities. For producers purchasing from another flock, the risk of acquiring infection also increases with consignment size.

### 6.2.2 Risk assessment scenarios

Risk assessments were undertaken for a number of scenarios, based on flocks classified according to State and Zone of origin, whether the flock was known to be infected or not and flock classification in the market assurance program. For known infected flocks, scenarios were run for high and low-prevalence flocks, based on the number of faecal pools culture-positive in a standard pooled-faecal-culture test of seven faecal pools of faeces each containing faeces from 50 sheep, and for flocks with 1 – 3 negative pooled-faecal-culture tests. The specific scenarios evaluated were as shown in Table 6.1. For the initial analysis flocks were assumed to be unvaccinated. A second analysis was also undertaken for the same scenarios, except that flocks were assumed to have been vaccinating all lambs born for the previous 2 – 6 years.
6.2.3 Calculating a risk-index

For each scenario considered, a risk-index for the scenario was calculated, as the estimated probability that a randomly selected animal from a randomly selected flock would be infected. Risk-indices were calculated for groups of flocks sharing similar characteristics because the intention was to group flocks according to the risk they pose, based on geographic, management and disease-related factors. The risk associated with movements of sheep between regions were not considered because there was insufficient data on sheep movements and trading patterns to support such an analysis.

Table 6.1. Summary of risk-assessment scenarios based on State and Zone of origin, flock status and testing history

<table>
<thead>
<tr>
<th>Zone or flock type</th>
<th>State/Status</th>
</tr>
</thead>
</table>
| Protected Zone     | Queensland unrestricted\(^a\)  
|                    | South Australia unrestricted 
|                    | Tasmania unrestricted 
|                    | New South Wales unrestricted 
|                    | Victoria unrestricted 
| CT\(^b\)           | MN2\(^c\) 
|                    | MN3\(^d\) |
| Control Zone       | Unrestricted 
|                    | CT 
|                    | MN1\(^e\) 
|                    | MN2 
|                    | MN3 |
| Residual Zone      | Unrestricted 
|                    | CT 
|                    | MN1 
|                    | MN2 
|                    | MN3 |
| Infected flocks    | High prevalence\(^f\) 
|                    | Low prevalence\(^g\) 
|                    | 1 negative test 
|                    | 2 negative tests 
|                    | 3 negative tests |

\(^a\) Unrestricted flocks are flocks which can trade freely within the zone or State without restrictions  
\(^b\) CT = Check tested — Pooled faecal culture of 2 pools of 50 sheep or serology on 250 sheep with negative results within the last 12 months,  
\(^c\) MN2 = Monitored Negative 2 status in the SheepMAP,  
\(^d\) MN3 = Monitored Negative 3 status in the SheepMAP,  
\(^e\) MN1 = Monitored Negative 1 status in the SheepMAP,
High prevalence = ≥3 pools positive out of 7 pools of 50 tested by pooled faecal culture,
Low prevalence = 1 or 2 pools positive out of 7 pools of 50 tested by pooled faecal culture.

6.2.4 Model structure

The basic risk-index for a flock or group of flocks sharing similar characteristics was calculated as:

Risk-index = FP * WFP * STATE * VACC

where FP and WFP were the assumed values for flock-prevalence and within-flock prevalence, respectively, STATE was the assumed relative change in FP associated with State of origin for flocks in the Protected Zone and VACC was the assumed relative change in WFP in vaccinated flocks. Assumed values for FP, WFP STATE and VACC were specified for each scenario as described in Section 6.2.5, below.

The above formula for risk-index was implemented in a simulation model using an Excel® spreadsheet and @Risk® simulation software. For each iteration of the model, the risk-index was equivalent to the risk of infection for a single, randomly selected adult sheep from a randomly selected flock.

6.2.5 Model inputs

Beta distributions were used to describe FP and WFP input values, because this is a commonly used distribution for expressing uncertainty about a proportion (such as prevalence) and parameters can be easily determined from the mode and 5th or 95th percentiles (Vose, 1997 Vose, 2000, p 104; Suess EA et al., 2002). Estimates of the most likely value (mode) and the 5th or 95th percentiles of possible values for FP and
WFP were determined by agreement among a group of four experts. Where the estimated mode was \( \leq 0.5 \), the mode and 95th percentile were used to determine distribution parameters, and where the estimated mode was \( > 0.5 \), the mode and 5th percentile were used. The FreeBS computer program (Su et al., 2002) was used to estimate alpha and beta parameters for the resulting beta distributions of FP and WFP. The assumed most likely values and 95% limits for input distributions for FP and WFP for each scenario are shown in Table 6.2, along with alpha and beta values that best fit the assumed values.

Pert distributions were used for STATE and VACC because this is a useful distribution for expressing uncertainty about a value, based on expert opinion as to its minimum, most likely and maximum values (Vose, 2000, p 105). Minimum, most likely and maximum values for STATE and VACC are shown in Table 6.3. Values for STATE were based on previous estimates of flock-prevalence by State and region, calculated from abattoir surveillance data up to 31 December 2001 (Sergeant, 2003, in press – see Appendix). Assumed values for FP for unrestricted flocks from Queensland were as shown in Table 6.2 for the ‘Protected Zone – unrestricted’ category. Most likely values for relative risk for other states were calculated as the ratio between the most likely value FP for that State, as shown in Table 6.3, and the most likely value of FP for Queensland. Maximum values were calculated as the ratio between the assumed maximum FP for each State and the most likely value of FP for Queensland. The minimum relative risk for all States was assumed to be one and STATE for Control and Residual Zones also was set to equal one.
Table 6.2. Prior estimates of mode and 95% limit for FP and WFP for various combinations of zone and flock classification, and alpha and beta values for the input distributions for each parameter.

<table>
<thead>
<tr>
<th>Zone and Flock Status</th>
<th>Parameter</th>
<th>Most likely value (%)</th>
<th>Upper/ lower 95% limit (%)</th>
<th>Alpha</th>
<th>Beta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protected zone</td>
<td>FP</td>
<td>0.1</td>
<td>0.5</td>
<td>2</td>
<td>920</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>1.0</td>
<td>5.0</td>
<td>1.9</td>
<td>88</td>
</tr>
<tr>
<td>CTb</td>
<td>FP</td>
<td>0.5</td>
<td>1.0</td>
<td>6.7</td>
<td>1138</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>1.0</td>
<td>2.0</td>
<td>6.6</td>
<td>560</td>
</tr>
<tr>
<td>MN2c</td>
<td>FP</td>
<td>0.2</td>
<td>0.5</td>
<td>5</td>
<td>1838</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>0.7</td>
<td>2.0</td>
<td>3.4</td>
<td>338</td>
</tr>
<tr>
<td>MN3d</td>
<td>FP</td>
<td>0.1</td>
<td>0.50</td>
<td>1.9</td>
<td>920</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>0.7</td>
<td>1.5</td>
<td>5.7</td>
<td>664</td>
</tr>
<tr>
<td>Control zone</td>
<td>FP</td>
<td>10.0</td>
<td>20.0</td>
<td>5.7</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>2.0</td>
<td>10.0</td>
<td>1.8</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>7.5</td>
<td>15.0</td>
<td>6</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>2.2</td>
<td>5.4</td>
<td>4.2</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>MN1e</td>
<td>5.0</td>
<td>10.0</td>
<td>6.2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>1.0</td>
<td>2.7</td>
<td>3.6</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td>MN2</td>
<td>0.70</td>
<td>1.5</td>
<td>5.7</td>
<td>664</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>0.80</td>
<td>1.7</td>
<td>5.8</td>
<td>593</td>
</tr>
<tr>
<td></td>
<td>MN3</td>
<td>0.15</td>
<td>0.5</td>
<td>2.8</td>
<td>1190</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>0.80</td>
<td>1.4</td>
<td>9.8</td>
<td>1090</td>
</tr>
<tr>
<td>Residual zone</td>
<td>FP</td>
<td>40.0</td>
<td>55.0</td>
<td>12.7</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>5.0</td>
<td>30.0</td>
<td>1.5</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>15.0</td>
<td>30.0</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>3.3</td>
<td>8.0</td>
<td>4.1</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>MN1</td>
<td>10.0</td>
<td>20.0</td>
<td>5.7</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>1.2</td>
<td>4.0</td>
<td>2.7</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>MN2</td>
<td>2.00</td>
<td>4.0</td>
<td>6.5</td>
<td>272</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>1.0</td>
<td>2.0</td>
<td>6.6</td>
<td>560</td>
</tr>
<tr>
<td></td>
<td>MN3</td>
<td>0.60</td>
<td>1.20</td>
<td>6.7</td>
<td>945</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>1.0</td>
<td>1.6</td>
<td>13.4</td>
<td>1233</td>
</tr>
<tr>
<td>Infected flocks</td>
<td>FP</td>
<td>100.0</td>
<td>100.0</td>
<td>10000</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>10.0</td>
<td>30.0</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>100.0</td>
<td>100.0</td>
<td>10000</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>1.5</td>
<td>3.0</td>
<td>6.5</td>
<td>370</td>
</tr>
<tr>
<td></td>
<td>1 negative test</td>
<td>95.0</td>
<td>80.0</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>0.49</td>
<td>2.1</td>
<td>2.3</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td>2 negative tests</td>
<td>80.0</td>
<td>50.0</td>
<td>7.5</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>0.49</td>
<td>2.1</td>
<td>2.3</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td>3 negative tests</td>
<td>50.0</td>
<td>30.0</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>WFP</td>
<td>0.49</td>
<td>2.1</td>
<td>2.3</td>
<td>260</td>
</tr>
</tbody>
</table>
Unrestricted flocks are flocks which can trade freely within the zone or State without restrictions.

CT = Check tested — Pooled faecal culture of 2 pools of 50 sheep or serology on 250 sheep with negative results within the last 12 months,

MN2 = Monitored Negative 2 status in the SheepMAP,

MN3 = Monitored Negative 3 status in the SheepMAP,

MN1 = Monitored Negative 1 status in the SheepMAP,

High prevalence = ≥3 pools positive out of 7 pools of 50 tested by pooled faecal culture,

Low prevalence = 1 or 2 pools positive out of 7 pools of 50 tested by pooled faecal culture.

Table 6.3. Assumed values for the median and 95th percentile for FP by STATE and for minimum, most likely and maximum values for the relative risk values for STATE and VACC.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Level</th>
<th>Assumed value for FP</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median (%)</td>
<td>95th percentile (%)</td>
</tr>
<tr>
<td>STATE</td>
<td>Queensland</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>South Australia</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Tasmania</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>New South Wales</td>
<td>0.8</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Victoria</td>
<td>1.8</td>
<td>3.5</td>
</tr>
<tr>
<td>VACC</td>
<td>&lt;=2 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;2 to &lt;6 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;=6 years</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Simulation modelling was used to estimate values for VACC — the relative risk of infection for vaccinated adults compared to unvaccinated adults under the same conditions. Briefly, a stochastic simulation model was used to estimate infection-prevalence (Sergeant, 2002a; Sergeant, 2002b), with input parameters specified that resulted in a median annual mortality rate in adults of 12% per year (95% interval: 10.5 – 13.5%). The model was run for two scenarios, both for a simulated 30-year period, for an assumed self-replacing flock of 2,000 ewes, with four infected animals in year 0. The
first scenario assumed that no control measures were implemented, while the second
assumed that annual vaccination of lambs commenced in year 20 and continued until
the end of the simulation period. The vaccination scenario assumed a moderate vaccine
efficacy, with 60% of susceptible vaccinates becoming resistant to infection and a 40%
reduction in the rate of progression of latent infections in vaccinated animals. These
assumed values produce simulation results that are consistent with the observed effect
of vaccination in preliminary results from a vaccination trial in New South Wales
(Eppleston et al., 2002). For each scenario, the proportion of infected adults was
calculated at the end of each simulated year, and the ratio between prevalence in
vaccinated and unvaccinated adults was calculated. In this analysis, prevalence in
vaccinated adults averaged about 0.5 times (range 0.2 – 0.7) the prevalence in
comparable non-vaccinated adults in the period from 2 – 6 years after commencing
vaccination and was <0.2 times prevalence in non-vaccinates for flocks vaccinating for
>6 years. Based on these estimates, the assumed values for the minimum, most likely
and maximum values for VACC were 0.2, 0.5 and 0.7, respectively.

6.2.6 Model outputs

For each iteration of the risk-model, new values were selected from each of the input
distributions, and a new value for the risk-index was calculated. The model was run for
5,000 iterations for each scenario, to generate a distribution of risk-index values. For
each scenario, the mean and 95th percentile of the output distribution were reported.

6.2.7 Calculating a risk-score

For each scenario, a risk-score was calculated from the mean risk-index value as Risk-
score = \log_2(\text{risk-index} \times 10000). The risk-score was rounded to the nearest integer, and
was re-scaled to provide a minimum value of 0. The resulting scale of risk-scores was a series of integer values, with each unit increase or decrease in score representing a doubling or halving respectively of the risk compared to the previous category. A log$_2$ scale was used to transform the risk-index because it provides a better discrimination between adjacent risk-categories and allows a more meaningful and intuitive interpretation of risk-score values than would a simple linear transformation or alternative log-values.

### 6.3 Results

The base risk-scores ranged from 0 (lowest risk) to 13 (highest risk), corresponding to risk-index values of 0.00002 to 0.14 (Table 6.4). The risk-scores for the upper 95$^{\text{th}}$ percentile were 1 – 2 points higher than the mean, but generally had the same ranking, while vaccination resulted in a one-point reduction in the risk-score for all scenarios. Scenarios were grouped by risk-score as shown in Table 6.5, to facilitate comparison between categories.

### 6.4 Discussion

The approach described above provides a useful framework for objectively assessing risk of OJD infection based on flock characteristics and for comparison of risk between flocks with different characteristics. The integer scale used also makes it relatively simple to understand and use.
Table 6.4. Mean and upper 95% values for risk-index for various base-scenarios, and for corresponding scenarios assuming vaccination of lambs has been practised for 2 – 6 years, and mean values calculated using a deterministic approach, sorted by zone and State/status

<table>
<thead>
<tr>
<th>Zone or flock type</th>
<th>State/Status</th>
<th>Base scenario</th>
<th>95%</th>
<th>Vaccination scenario</th>
<th>95%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Risk-index</td>
<td>Risk-score</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protected Zone</td>
<td>Queensland unrestricted</td>
<td>0.00005</td>
<td>2</td>
<td>0.00014</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>South Australia unrestricted</td>
<td>0.00010</td>
<td>3</td>
<td>0.00032</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Tasmania unrestricted</td>
<td>0.00017</td>
<td>4</td>
<td>0.00056</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>New South Wales unrestricted</td>
<td>0.00044</td>
<td>5</td>
<td>0.00146</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Victoria unrestricted</td>
<td>0.00082</td>
<td>6</td>
<td>0.00276</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>0.00007</td>
<td>2</td>
<td>0.00014</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>MN2</td>
<td>0.00003</td>
<td>1</td>
<td>0.00007</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>MN3</td>
<td>0.00002</td>
<td>0</td>
<td>0.00005</td>
<td>2</td>
</tr>
<tr>
<td>Control Zone</td>
<td>Unrestricted</td>
<td>0.00477</td>
<td>9</td>
<td>0.0126</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>0.00250</td>
<td>8</td>
<td>0.0055</td>
<td>9</td>
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<td></td>
<td>MN1</td>
<td>0.00079</td>
<td>6</td>
<td>0.0018</td>
<td>7</td>
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<td>0.00008</td>
<td>3</td>
<td>0.0002</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>MN3</td>
<td>0.00002</td>
<td>1</td>
<td>0.0000</td>
<td>2</td>
</tr>
<tr>
<td>Residual Zone</td>
<td>Unrestricted</td>
<td>0.0486</td>
<td>12</td>
<td>0.1226</td>
<td>13</td>
</tr>
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<td></td>
<td>CT</td>
<td>0.0072</td>
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<td>0.0160</td>
<td>10</td>
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<td>MN1</td>
<td>0.0022</td>
<td>7</td>
<td>0.0052</td>
<td>9</td>
</tr>
<tr>
<td></td>
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<td>0.0003</td>
<td>4</td>
<td>0.0006</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>MN3</td>
<td>0.0001</td>
<td>3</td>
<td>0.0001</td>
<td>3</td>
</tr>
<tr>
<td>Infected flocks</td>
<td>High prevalence</td>
<td>0.143</td>
<td>13</td>
<td>0.297</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Low prevalence</td>
<td>0.017</td>
<td>10</td>
<td>0.030</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1 negative test</td>
<td>0.008</td>
<td>9</td>
<td>0.018</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2 negative tests</td>
<td>0.006</td>
<td>9</td>
<td>0.015</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>3 negative tests</td>
<td>0.004</td>
<td>8</td>
<td>0.010</td>
<td>10</td>
</tr>
</tbody>
</table>

*Unrestricted = flock not known to be infected and not either CT or in the SheepMAP,
CT = Check tested — Pooled faecal culture of 2 pools of 50 sheep with negative results within the last 12 months,
MN2 = Monitored Negative 2 status in the SheepMAP,
MN3 = Monitored Negative 3 status in the SheepMAP,
MN1 = Monitored Negative 1 status in the SheepMAP,
High prevalence = ≥3 pools positive out of 7 pools of 50 tested by pooled faecal culture,
Low prevalence = 1 or 2 pools positive out of 7 pools of 50 tested by pooled faecal culture.*
Table 6.5. Summary of flock types for each of the flock risk-score categories

<table>
<thead>
<tr>
<th>Risk-score</th>
<th>State/Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>MN3 in Protected Zone</td>
</tr>
<tr>
<td>1</td>
<td>MN3 in Control Zone, MN2 in Protected Zone</td>
</tr>
<tr>
<td>2</td>
<td>Queensland unrestricted(^a), CT(^b) in Protected Zone</td>
</tr>
<tr>
<td>3</td>
<td>MN3(^d) in Residual Zone, MN2 in Control Zone, South Australia unrestricted</td>
</tr>
<tr>
<td>4</td>
<td>Tasmania unrestricted, MN2 in Residual Zone</td>
</tr>
<tr>
<td>5</td>
<td>New South Wales unrestricted</td>
</tr>
<tr>
<td>6</td>
<td>MN1(^e) in Control Zone, Victoria unrestricted</td>
</tr>
<tr>
<td>7</td>
<td>MN1 in Residual Zone</td>
</tr>
<tr>
<td>8</td>
<td>CT in Control Zone, 3 negative tests</td>
</tr>
<tr>
<td>9</td>
<td>Unrestricted in Control Zone</td>
</tr>
<tr>
<td>9</td>
<td>Infected flock with 2 negative tests, Infected flock with 1 negative test, CT in Residual Zone</td>
</tr>
<tr>
<td>10</td>
<td>Infected flock with low prevalence(^g)</td>
</tr>
<tr>
<td>12</td>
<td>Unrestricted in Residual Zone</td>
</tr>
<tr>
<td>13</td>
<td>Infected flock with high prevalence(^f)</td>
</tr>
</tbody>
</table>

\(^a\) Unrestricted = flock not known to be infected and not either CT or in the SheepMAP,
\(^b\) CT = Check tested — Pooled faecal culture of 2 pools of 50 sheep with negative results within the last 12 months,
\(^c\) MN2 = Monitored Negative 2 status in the SheepMAP,
\(^d\) MN3 = Monitored Negative 3 status in the SheepMAP,
\(^e\) MN1 = Monitored Negative 1 status in the SheepMAP,
\(^f\) High prevalence = \(\geq 3\) pools positive out of 7 pools of 50 tested by pooled faecal culture,
\(^g\) Low prevalence = 1 or 2 pools positive out of 7 pools of 50 tested by pooled faecal culture.

For simplicity it may be appropriate to cap the scale with a maximum value (for example 10 or 12) and adjust the individual values slightly to ensure appropriate grouping of flocks with similar risk and adequate separation of those with different risks. Considering the uncertainty about the risk-estimates provided here, manual refinement of the risk-scores for individual categories is not unreasonable. If necessary, it would also be possible to further reduce the number of categories by either re-analysing using a different log scale, or simply by amalgamating groups with similar scores. However, this would result in a loss of discrimination across the spectrum of risk, and would also make the adjustment of risk-score for risk/mitigating factors more difficult.
The estimates of WFP used in this analysis were derived by expert opinion, and therefore are subject to uncertainty and potential bias. However, the estimates were agreed by a panel of experts and were the best estimates available for this analysis. In addition, because of the conversion to an integer score, rather than using a true quantitative risk value, the absolute magnitude of the values is of lesser importance than the relative differences between categories. Similarly, input values used for FP, VACC and STATE may also be subject to error. The impact of any error was reduced by the use of probability distributions rather than fixed values in the risk-model. This allowed the generation of a probability distribution for the resulting risk-scores, reflecting any uncertainty about the true values for the various input parameters. Importantly, the resulting risk-scale appeared to rank the various classifications appropriately, with no classifications that were obviously incorrectly ranked.

An important feature of this approach is that additional risk or mitigating factors can be easily incorporated in the risk assessment by adjusting the risk-score upwards or downwards according to the estimated relative risk associated with the factor. Because the risk-score is calculated on a log₂ scale, each point on the scale represents a two-fold increase or decrease in risk. Therefore, each doubling or halving of the relative risk for a factor results in a one-point addition or deduction respectively to the score. For example, the modal value for relative risk associated with vaccination for 2 – 6 years was 0.5, corresponding to a one point reduction risk-score, which is the same result as was demonstrated by simulation. Therefore, instead of including vaccination explicitly in the risk-model, risk-scores could have been adjusted post-hoc for the assumed effect of vaccination. Alternatively, if the relative risk associated with having one infected
neighbour was assumed to be four, this corresponds to a two log_2 increase in risk, or a
two point increase in risk-score.

Using this approach, a wide variety of risk or mitigating factors could be considered,
including: negative surveillance results; implementation of biosecurity measures;
presence and number of infected neighbours; purchasing history; or measures taken to
reduce prevalence, such as vaccination or grazing management. For any factor to be
included, it would be necessary to determine appropriate categories for the factor and an
estimate of the likely relative risk of infection for each category, relative to the default
value. This method for adjusting risk-scores depends on two important assumptions —
that putative risk or mitigating factors are independent of each other and that the factors
impact uniformly across States, zones and for varying flock-classifications. Given the
nature of the factors suggested, and the variation between the various flock categories,
these assumptions are unlikely to be met and the resulting risk-estimates will be
somewhat biased, with the nature and extent of the bias dependent on the type and
degree to which the assumptions were violated. However, estimation of stratum-specific
estimates is likely to be even more difficult, and the resulting model would be
considerably more complicated. Therefore, the approach outlined above appears to be
the most suitable option available based on our present understanding of OJD. Although
some potential risk and mitigating factors for OJD-risk have been identified, a full
analysis for each of these factors, singly or in combination has not been undertaken.
However, using the method described above, once estimates of the relative risks for
each category of these factors are available it is possible to adjust the risk-scores
accordingly.
Consignment size is also an important determinant of risk when purchasing sheep, although it has not been specifically considered in this analysis. In fact, as the number of sheep in a consignment increases, the risk of infection also increases in a non-linear fashion (for example, purchasing one sheep from an unrestricted flock in the South Australian Protected Zone (risk-score = 3) has a risk of about 0.0001 (1 in 10,000) of infection. Purchasing 10 or 100 sheep instead of one increases the risk of infection by 9-fold and 36-fold, respectively. Therefore, when developing a risk-based trading scheme, consignment size is an important consideration. Consignment size could be included as another risk factor, with the consignment-risk calculated by adjustment of the flock-risk, as described above. Alternatively, if it was not included in the risk-score, it would be important for purchasers to consider the effect of consignment size on the risk of acquiring infection.

An alternative quantitative approach, based on the crude risk-index value or on a different risk value calculated by alternative means (eg including consignment size) could be considered and would provide more information to producers on which to base decisions. However, because this alternative approach would provide risk values that may lie anywhere on a continuum of risk, the level of detail and complexity of such a system is likely to preclude its effective uptake by producers. Such a system would also be more difficult to adjust risk-estimates for additional risk or mitigating factors, as discussed above.

The system for risk-based classification of flocks discussed here has an intuitive appeal for further development and application in Australia’s national OJD program. The proposed system has the advantages of being both relatively simple and easy to
understand, but at the same time has a scientific basis. Because precise quantitative estimates of risk were not required, it was possible to convert the results of the detailed analysis to a simple numerical scale for each flock, greatly simplifying the resulting risk-score.

The main disadvantages are that the true value of many of the parameters used in developing a risk profile for any flock category are still unknown and so estimates must be based on expert opinion. Similarly, some of the assumptions on which the methodology has been based may not be valid, resulting in bias of the resulting risk-estimates. However, compared to the alternative of either rigorous risk assessments based on individual circumstances or a totally qualitative assessment of risks, it was felt that this approach provided a reasonable compromise, resulting in reasonable risk estimates that could be easily understood and interpreted by producers and their advisers for comparative purposes.

This approach provides a simple framework on which to develop a risk-based trading system for OJD. However, before this can be implemented it is essential to achieve agreement on the level of regulatory support required, as well as final risk-scores for each flock category and relative risk values for any risk factors included. Implementation of this approach will also depend on continuing availability of surveillance and other data to maintain the credibility of the proposed flock-categorisation.
Part 4

Discussion
7. Discussion

7.1 Introduction

When the NOJDP commenced in 1998, the number of known infected flocks and the geographic spread of infection were increasing (see Chapter 2). Despite this, the disease was thought to still have a highly clustered distribution, and it was hoped that it could be contained and that eventual control or eradication might have been possible. At that time, however, there was no objective data on the occurrence of the disease across Australia. Further, there was only a limited understanding of the epidemiology of this disease in Australia, and serology using the AGID was the only test available for flock-screening. Given this context, the NOJDP was planned as a six-year program directed at reducing the spread of OJD while undertaking research to assist in decision-making at the completion of the program in June 2004 (Allworth and Kennedy, 2000).

The research presented in this thesis has been an important component of the NOJDP, and has been instrumental in the ongoing refinement of the approach to OJD control and the consideration of future options for OJD control after the completion of the NOJDP.

7.2 Screening tests for OJD

Control of OJD in Australia has depended heavily on both surveillance testing, for the detection of infected flocks, and assurance testing, for the identification of low-risk flocks as a source of replacement sheep. When the NOJDP commenced, all testing was based on the AGID, with an assumed sensitivity of 30% (see Chapter 3). Pooled faecal culture and an absorbed ELISA were subsequently developed as alternative tests that were potentially more sensitive than the AGID, allowing potential reductions in
required sample sizes, and hence testing costs (see Chapters 3 & 4). However, before these new tests could be effectively used for surveillance or assurance testing, further evaluation was required to determine their sensitivity and specificity, and to determine how these tests could best be applied within the NOJDP.

An evaluation of an absorbed ELISA has shown that it had a similar sensitivity to the AGID (22% compared to 24%), but that the specificity was lower (99% compared to 100% in the panel of sera assessed) (see Chapter 3). Although the sensitivity of this ELISA could be improved to about 40% by reducing the cut-point value used to determine a positive result, this would also result in a consequent decrease in ELISA specificity to 95%. Based on these results, the ELISA is unlikely to be a useful screening test for OJD because of the unacceptably high false-positive rate.

This study also allowed an improved understanding of the performance of the AGID in Australian flocks. Importantly, the weighted-average AGID sensitivity across six infected flocks was only 24%, not 30% as previously assumed. Because sensitivity was estimated in comparison with histology, this estimate is likely to overestimate the true value to an unknown extent, because histology also has an imperfect sensitivity. In addition, AGID sensitivity was shown to vary significantly depending on lesion type, body condition-score and flock of origin. Sensitivity was highest in sheep with severe multibacillary lesions, and was lowest in sheep with paucibacillary lesions, regardless of lesion severity. Sensitivity was also highest in thin sheep, and decreased as sheep condition score increased. Both condition-score and flock effects remained significant after controlling for potential confounding by lesion type, suggesting that there might
have been other factors operating that affect AGID sensitivity, or that some misclassification of lesion type could have occurred.

In another study (Chapter 4), pooled faecal culture and serological testing using the AGID were compared as flock-screening tests for the detection of OJD. Using matched serological and faecal samples, the study found that the flock-sensitivity of pooled faecal culture was significantly higher than for AGID testing. The difference was particularly apparent in low-prevalence flocks (estimated true prevalence <2%), with pooled faecal culture detecting 82% of such flocks, compared to only 33% for the AGID, compared to 96% and 85% respectively for higher prevalence flocks.

Pooled faecal culture was found to be a cheaper and more sensitive test than the AGID, and is now the preferred test for all surveillance and assurance testing as part of the NOJDP. In addition, because of the difference in test sensitivities, the required sample sizes for serological testing have been increased approximately 2.5 fold (in comparison to pooled faecal culture), to achieve similar flock-level sensitivity.

7.3 Prevalence and distribution of OJD in Australia

One of the key objectives of the NOJDP was to estimate the prevalence and geographic distribution of OJD in Australia (Chapter 2). The introduction of abattoir surveillance as a broad-scale flock-screening tool in late 1999 offered the opportunity to address this key objective.

Prior to the introduction of abattoir surveillance, surveillance for OJD relied primarily on the identification of flocks regarded as being ‘at-risk’ of infection because of trading
links with known infected flocks or because they were neighbours of known infected flocks. As a consequence, surveillance activity was strongly biased. Furthermore, in the absence of identified connections with known infected flocks, many regions and flocks remained untested. This approach is in contrast to abattoir surveillance, which does not target particular flocks or regions, and therefore is able to capture information on flocks and regions that had not previously been subject to surveillance.

Despite the biases inherent in using abattoir data for estimating prevalence, this was thought to be the only feasible data on which to estimate the regional prevalence of OJD in Australia. In addition to the biases associated with the non-random nature of sampling through an abattoir, one important source of bias was the assumed imperfect flock-sensitivity of abattoir surveillance for the detection of infected flocks. To overcome this, the flock-sensitivity of abattoir surveillance under varying assumed conditions of flock-prevalence and within-flock prevalence was estimated using a simulation model. Flock-prevalence of OJD was then estimated on a State and regional (for New South Wales only) basis from abattoir surveillance results, using a Bayesian approach to adjust for the imperfect flock-sensitivity of abattoir surveillance (Chapter 5).

This analysis confirmed that the majority of infected flocks were located in central and southern New South Wales, with additional foci of infection on Kangaroo Island (South Australia) and Flinders Island (Tasmania). Estimated prevalence in other areas of Australia was generally low (<1%), except for Victoria, where the median estimated prevalence was about 1.8%. These results fulfilled a primary objective of the NOJDP, and at the same time provided a clearer definition of the distribution of OJD than had
previously been possible. Following this analysis, and supported by the abattoir surveillance results, most of the low-prevalence regions of south-east Australia were declared as Protected Zones for OJD during 2002.

7.4 Assessment of flock-risk for OJD

The final part of the current research has been to develop a method for assessing flock-risk for OJD, to support the development of future trading options based on individual flock-risk, rather than geographic risk and testing history alone (Chapter 6). A simple quantitative risk model allowed the development of a simple but objective risk-score, based on estimated flock-prevalence and within-flock prevalence for different classes of flocks, depending on their location and status. A method is also described for modifying this risk-score for individual flocks, according to the presence and level of individual flock risk factors such as the use of vaccination, surveillance history and the presence and number of infected neighbours. This allows individual producers to take steps to reduce the risk posed by their flock to other producers and to demonstrate the increased level of assurance associated with this reduced risk. This flock-based approach to risk assessment for OJD could be supported by varying degrees of regulatory control over sheep movements, or could be adapted to a deregulated environment, with sheep producers taking responsibility for their own risk management, rather than the State or Territory regulatory authorities.

This risk-assessment approach is now being used as the basis for a new approach to managing sheep movements, based on varying levels of flock-assurance. The approach will also encourage producers to be pro-active in managing risk, and to take positive steps to improve the level of assurance of their own flock.
7.5 Future directions in OJD control

Since the mid 1990’s, control of OJD has relied primarily on regulation, either through quarantine of known or suspected infected flocks, or by application of restrictions on the movement of sheep from higher prevalence to lower prevalence zones. While these measures have had some impact on the spread of disease, they have not been completely effective in preventing spread and have contributed to the negative financial and social effects of OJD on affected producers.

Therefore, as the current NOJDP is scheduled to wind up in June 2004, the opportunity has been taken to reconsider the future direction for any national program for the management of OJD. Consultations with government and sheep-industry representatives have resulted in the development of a “Future directions” discussion paper, which sets out a number of options for the future management of OJD in Australia (Anonymous, 2003). Further consultations will be undertaken to determine the final preferred option for future management of OJD in Australia.

Regardless of the detail of the final option selected, it appears likely that there will be a move away from a strong regulatory focus to one of producers taking responsibility for managing the disease themselves, possibly with some regulatory support. This will be supported by an extensive communication and education program to help producers understand the risks of OJD and how to manage their flock to reduce the risk and impact of infection. Any national program will also include ongoing research into the epidemiology, diagnosis and control of OJD.
While such an approach is also unlikely to achieve complete control of the disease, it will allow producers to manage their own risk, according to their circumstances, and will alleviate the significant financial cost imposed on affected producers by the current regulatory program. Under this approach, effective control of OJD will only be achieved if producers perceive that it is worth implementing measures to prevent its spread.

### 7.6 Conclusion

The results of this research have been very important for the NOJDP and for the future control of OJD in Australia. We now have a much better understanding of the performance of serology and pooled faecal culture for the detection of infected animals and flocks, and of the level of assurance that is achieved by negative testing than was the case previously. In addition, we have an objective assessment of the distribution and likely prevalence of OJD in Australia, and a useful tool for monitoring the prevalence of infected flocks on an ongoing basis. Finally, the development of a simple, objective framework for assessing flock-risk of OJD will allow a move away from the previous highly regulated management of the disease, to a situation with less emphasis on regulation and increased emphasis on producers managing their own risk.
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9. Appendix

Estimated flock-prevalence and distribution of ovine Johne’s disease in Australia at December 2001

ESG SERGEANT

AusVet Animal Health Services, 69 Turner Crescent, Orange NSW 2800

As part of the National Ovine Johne’s Disease Control and Evaluation Program, the flock-prevalence and distribution of ovine Johne's disease (OJD) in Australia at the end of December 2000 were recently estimated using data from abattoir surveillance and other sources. A Bayesian method was used to adjust for the imperfect sensitivity of abattoir surveillance and for uncertainty about sensitivity and assumed flock-prevalence in each State and region.

This report updates the previous estimates of OJD flock-prevalence by State, and by region within New South Wales. The same method as for the previous analysis was used, with flock-prevalence estimates updated to take into account the results of abattoir surveillance undertaken during 2001.

For the current analysis, the same prior distributions for the sensitivity and specificity of abattoir surveillance were used as for the previous analysis for each State or region. α and β parameters for prior distributions for flock-prevalence were estimated by trial-
and-error, to provide distributions that closely approximated the median and 97.5\textsuperscript{th} percentile of the output distributions for flock-prevalence from the previous analysis. The results of abattoir surveillance for the 12 months to 31 December 2001 for each State or region were used as inputs. A Gibbs sampler was used to combine the prior flock-prevalence, sensitivity and specificity estimates with the observed results of abattoir surveillance, to produce probability distributions of flock-prevalence for each State and region. Estimates are presented as median and 95\% probability intervals from the probability distributions.

For the analysis of New South Wales’ data, the State was divided into three regions according to apparent prevalence of known infected flocks (at 31 December 2000) by Rural Lands Protection Board as follows:

High Prevalence (HP): >5\% of flocks infected – Central Tablelands and Goulburn RLPB districts

Moderate Prevalence (MP): 0.5 – 5\% of flocks infected - Wagga Wagga, Murray, Forbes, Cooma, Gundagai, Hume, Young, Braidwood, Molong and Yass RLPB districts

Low Prevalence (LP): <0.5\% of flocks infected – Remaining RLPB districts

For other States, prevalence was estimated for the entire State, except for Tasmania, where data for Flinders Island were excluded from the analysis.

The results of this analysis are summarised in Table 1. For Queensland, South Australia, Tasmania and Western Australia the estimated prevalence remained virtually unchanged. Median prevalence estimates in Victoria and the three regions of New South
Wales have all increased slightly from the 2000 estimates. This is associated with an increased proportion of lines from these areas that were positive in 2001 compared to 2000. These increases might reflect a real increase in the underlying prevalence due to ongoing spread of infection, or may be due to normal sampling variation and the effect of biases inherent in using abattoir surveillance as a tool to estimate flock-prevalence.

The estimates of flock-prevalence presented here are subject to a number of potential biases. Briefly, the estimates may be affected by the non-representative nature of abattoir surveillance as a sampling procedure for the population of interest, as well as by uncertainty about the true flock-sensitivity of abattoir surveillance and of prior estimates of flock-prevalence.

Because of the potential for bias, the estimates presented here must be regarded as approximate, rather than highly precise estimates of flock-prevalence. Despite this, these estimates provide the best available indicators of prevalence and distribution at this time. Importantly, the apparent distribution of OJD in Australia has remained largely unchanged after an additional 12 months and 20,000 lines of sheep subjected to abattoir surveillance. Therefore, although there may be some additional infected flocks still undetected in the low prevalence areas, there are unlikely to be major foci of infection still undetected.

Ongoing surveillance will allow these estimates to be further refined as more data becomes available. However, particularly in the lower prevalence regions, estimates are unlikely to change substantially without improved certainty about the true sensitivity of
abattoir surveillance in these areas, even with large increases in the amount of
surveillance undertaken.

Acknowledgments
This study was commissioned by Animal Health Australia as part of the National Ovine
Johne's Disease Control and Evaluation Program, using data provided by State
authorities.

References
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Table 1: Estimated flock-prevalence of ovine Johne’s disease in six States of Australia at 31 December 2000, plus abattoir surveillance results and updated flock-prevalence estimates to 31 December 2001

<table>
<thead>
<tr>
<th>State/Region</th>
<th>Estimated prevalence, December 2000</th>
<th>Abattoir surveillance results for 2001</th>
<th>Updated prevalence, December 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median 95% Interval</td>
<td>Lines tested</td>
<td>Lines +ve</td>
</tr>
<tr>
<td>NSW – HP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33 29 - 39</td>
<td>1137</td>
<td>403</td>
</tr>
<tr>
<td>NSW – MP&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11 8 - 15</td>
<td>3760</td>
<td>343</td>
</tr>
<tr>
<td>NSW – LP&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.5 0.05 - 1.5</td>
<td>4704</td>
<td>23</td>
</tr>
<tr>
<td>Qld</td>
<td>0.3 &lt; 1.0</td>
<td>1045</td>
<td>0</td>
</tr>
<tr>
<td>SA&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.15 0.03 - 0.5</td>
<td>3538</td>
<td>3</td>
</tr>
<tr>
<td>Tas&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.4 0.05 - 1.1</td>
<td>260</td>
<td>0</td>
</tr>
<tr>
<td>Vic</td>
<td>0.2 0.05 - 1.1</td>
<td>5143</td>
<td>54</td>
</tr>
<tr>
<td>WA</td>
<td>0.2 &lt; 0.8</td>
<td>566</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> HP: >5% of flocks confirmed infected – Central Tablelands and Goulburn Rural Lands Protection Board districts

<sup>b</sup> MP: 0.5 – 5% of flocks confirmed infected – Wagga Wagga, Murray, Forbes, Cooma, Gundagai, Hume, Young, Braidwood, Molong and Yass districts

<sup>c</sup> LP: <0.5% of flocks confirmed infected – 29 remaining Rural Lands Protection Board districts of New South Wales

<sup>d</sup> Data for Kangaroo Island were excluded from prevalence estimate at December 2000

<sup>e</sup> Data for Flinders Island were excluded from prevalence estimates December 2000 and December 2001