Longitudinal study of the spread of ovine Johne’s disease in a sheep flock in southeastern New South Wales, Australia

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ABSTRACT

On-farm investigation and monitoring for ovine Johne’s disease was performed in a flock of approximately 3000 sheep between 1997 and 2002. The study aimed to better understand ovine Johne’s disease prevalence, distribution and spread on this recently infected farm and to plan practical disease control and intervention strategies.

The AGID, pooled faecal culture (PFC) and histopathology were used for partial and then whole flock testing using PFC annually three times. Faecal shedding of Mycobacterium paratuberculosis (MAP) commenced in home bred sheep around six to seven years after a single introduction of a mob of 410 infected sheep in 1993. For at least seven years there was a clustering of infection and shedding within one or two age groups only. Sheep in these age groups appeared to have been exposed to mycobacterial contamination at an early age (<12 months) and commenced shedding at five years of age or older. Groups that were exposed to contamination as adults did not shed detectable levels of MAP during the study period.

These results provided indirect evidence of the finite duration of survival of MAP on pasture and the influence of age on susceptibility of sheep to developing patent MAP infection. A further feature of the epidemiology in this flock was the slow transmission of MAP, related to the long incubation period (exposure to shedding interval) of five years and the absence of clinical signs of OJD throughout the study period.

The findings suggest that management practices such as partial flock culling, selective grazing management and vaccination might have reduced mycobacterial contamination on this farm, possibly to a level at which patent MAP infection no longer occurred. Better understanding of disease spread within flocks over time through flock profiling using PFC will help devise surveillance strategies (including testing protocols for market assurance testing) that account for clustering of infection as well as very slow transmission of infection through a flock.

Key words: ovine Johne’s disease, clustering, whole flock testing, flock profiling, pooled faecal culture testing.

INTRODUCTION

OJD is a chronic wasting disease caused by Mycobacterium avium subsp. paratuberculosis (MAP). The major mode of transmission of bacteria is by the faecal-oral route. Once ingested, it usually takes at least 12 months before a patent infection establishes and detectable levels of bacteria are shed via faeces (Chaitaweesub et al, 1999). Appearance of clinical signs of chronic wasting usually takes many years (Whittington et al, 2001). The organism remains viable in the environment for up to about 12 months but infectious doses may be present for a shorter period (Whittington, 2001). Less is known about transmission of MAP in sheep than cattle, but calves are reported to be more susceptible to infection than mature cattle and the same might apply to sheep (Whittington et al, 2001).
There is very little objective information regarding the spread of MAP within an infected flock. It is assumed that most sheep are exposed via pasture, and that all animals in a flock have an equal chance of becoming exposed. Surveillance programs to detect infection entail sampling based on this assumption and ignore potential clustering of infections (Whittington et al, 2001). In addition, recommendations for control of OJD are based on whole flock destocking or quarantine and other trading restrictions to reduce further spread. These make no allowance for the possibility that infection is confined to subgroups or mobs within a flock. The availability of pooled faecal culture (Whittington et al, 2000) has enabled relatively inexpensive testing of whole flocks, to better determine the source and distribution of infection.

The aim of this study was to apply whole flock testing over time to determine the prevalence, distribution and spread of infection in a recently infected flock. This data may support the development of practical plans for farm intervention strategies to control the disease.

MATERIALS AND METHODS

The farm consisted of 1739 hectares of improved pasture located in southeastern New South Wales (NSW), a 600 mm average annual rainfall area. The main enterprise on this farm was sheep breeding, but around 700 beef cattle were also grazed and 250-300 hectares were cropped annually. The sheep flock consists of a commercial Bond breed flock of approximately 3000 breeding ewes and a Bond stud of about 300 ewes. Lambing is in April/May. Wether lambs are sold to slaughter from December onwards each year. Culls are sold to slaughter annually in September/October and usually include the entire oldest age group (six year olds). Prior to their first joining, the ewes are classed and around 50 are selected each year to enter the stud. Sheep are segregated based on age and kept in separate mobs, up to six years old. The commercial and stud ewes are kept separately. The mobs are grazed in a rotational pattern, fitting in with pasture availability and the cropping program. Other than the introduction of some Merino sheep (described below) and a single purchase of five Dorset rams in 1997 no sheep are routinely introduced.

In 1993 the owner purchased 410 mixed age fine wool Merino ewes from farm X, located in what is now recognized as the high prevalence OJD area in NSW. On the study farm the Merino sheep had no direct contact with the Bond flocks. OJD was confirmed on farm X in late 1996.

In early 1996 the owner of the study farm sold 287 of the Merino ewes to another farm (farm F) and the remainder to slaughter. Any remaining progeny from these Merino ewes were sold to slaughter in December 1996.

The 237 merino ewes on farm F were tested with AGID in October 1996 and there were nine positive results. Three of these sheep were examined at post mortem; histology on intestinal tissue showed lesions consistent with multibacillary Johne’s disease in all three sheep. The acid fast organisms in the formalin-fixed paraffin-embedded tissues of each sheep were identified as S strain MAP.

In March 2000, OJD was confirmed on the study farm in homebred 5-year old Bond ewes (see results). Whole flock testing of adult sheep by pooled fecal culture (PFC) commenced later in the year and was repeated annually three times in total. Methods for testing for OJD and further details are described in Rast and Whittington, 2005.

RESULTS

The patterns of testing and infection on the study farm are provided in Fig. 1 and Table 1.

Testing prior to 2000
Testing commenced on the study farm in 1997, as potentially infected sheep had been introduced from Farm X in 1993.
In 1997, 50 homebred 1992-born Bond sheep, which may have had contact with MAP-contaminated land, were tested using the AGID test with negative results.

In June 1998, 460 homebred Bond sheep, born in 1993 and 1994, were tested using the AGID and PFC, with negative results.

In March 2000, OJD was finally confirmed in 1995-born homebred Bond ewes, by histopathology following serology (AGID) tests of 450 sheep (Table 1, Fig. 1).

Testing was required on three occasions over a four-year period to confirm infection in homebred sheep. Formalin-fixed paraffin-embedded tissues from one sheep with multibacillary lesions were examined by PCR and were confirmed to contain S strain of MAP.

**Testing in 2000**

All sheep older than two years were tested with PFC in June 2000. Shedding of MAP was confirmed in the 1995-born commercial ewes (prevalence 2.5%). This was the age group known to be infected based on AGID tests with subsequent post mortem examination and histopathology testing, performed earlier in the same year. All results from other age groups were negative (Fig. 1 and Table 1).

**Testing in 2001**

All sheep older than 12 months were sampled for PFC in September 2001. Shedding of MAP was detected in the 1996-born commercial Bond ewes (prevalence 0.8%) and 1999-born stud ewes (prevalence 1.4%) (Fig. 1 and Table 1).

**Testing in 2002**

All sheep older than 12 months were tested using PFC in September-October 2002. Shedding was confirmed again in the 1996-born commercial ewes and prevalence had increased from 0.8% to 2.5%. The 1997-born commercial ewes were also positive (prevalence 0.7%). All other results were negative, including the 1999-born stud ewes that had tested positive the previous year (Table 1 and Fig. 1).

**Infection management on the study farm.**

After notification of infection on Farm X (source of putative infected Merino sheep) but prior to OJD confirmation on the study farm the owner identified the land used to graze the putative infected introduced Merino sheep and their progeny and ceased sheep grazing on this land in 1996. He applied lime at a rate of 2.5 tones per hectare and used the land for cattle grazing or cropping instead.

After OJD confirmation on the study farm in 2000, the first whole flock test by PFC in 2000 established that only the 1995-born sheep were shedding at detectable levels of MAP at the time of sampling. Faecal samples were collected in June, however the serology and histopathology results of multibacillary OJD from a limited number of sheep in that age group in March 2000 made it likely that they were shedding by then. Therefore lambs born to these ewes in April/May 2000 were at high risk of exposure and infection. A recommendation was made in 2000 to cull the entire 1995-born age group, including the lambs born to them. The owner sold all remaining 1995 born ewes (207 ewes) to slaughter in October 2000, and the lambs as they reached market weight between October and December 2000.

At that stage it was considered that the 1999-born sheep were potentially at high risk of infection through contamination from MAP shed by the infected 1995-born sheep in 2000 or earlier. The 1996 and 1997 born sheep were also considered at risk by exposure to contamination from the introduced Merino sheep (Fig. 1). However culling of other age groups considered at risk of infection was not an economically viable option.

It was recommended that land on which the confirmed infected and shedding 1995-born age group had been kept be used for purposes other than sheep grazing. The owner was able to identify and use the paddock these sheep had been in from the beginning of 1999 to late 2000 for cropping and cattle grazing in the following years.

In 2001, use of Gudair® vaccine was approved on the study farm and a vaccination program began. All ewe lambs born in 2001 and the 1999-born commercial and stud ewes were vaccinated at the time of faecal
sampling. Other age groups were not vaccinated due to the doubtful efficacy of vaccine administered post exposure to adults and the cost.

During 2002, as the test results from the 2001 sampling showed an apparent increase in shedding, all sheep (including adults) considered at risk but not already shedding were vaccinated. These were the 1998-, 2000- and 2002-born ewes and all rams.

DISCUSSION

Observations and test results support the following scenario: 1) Infection was introduced onto the study farm with Merino sheep purchased in 1993 and this source of contamination was removed in 1996; 2) Shedding in infected merino sheep occurred prior to their detection, and infective concentrations of MAP from the Merino sheep were present in the environment on the study farm from 1995 to 1997. This is based on a 12-month survival period on pasture after removal of the infected sheep (Whittington et al, 2004); 3) Only sheep less than 12 months old when exposed to MAP developed patent (progressed to shedding MAP) during the study period; 4) Shedding commenced when sheep were five years or older, a significantly longer time period than the shortest reported 12 months incubation period in sheep (Chaitaweesub et al, 1999).

The source of infection on the study farm was most likely environmental contamination with MAP from some of the 410 Merino sheep and their progeny introduced from Farm X in 1993 and sold in 1996. No other sources of infection are known. Although the Merino sheep were never tested while present on the study farm, these introduced Merino sheep were likely to be shedding MAP because 8 months after their sale to Farm F the prevalence of sero-positive sheep was 3.8%. Estimation of the true prevalence of infection from these results is problematic because of the reported extreme variation in sensitivity of the AGID (Sergeant et al, 2003; Sacks et al 1989). Shedding as early as 1995/1996 by these sheep can be assumed because the seropositive sheep examined on Farm F had multicellular intestinal lesions, a feature highly correlated with shedding of MAP in faeces (Whittington et al, 1999). Negative test results in the home-bred Bond sheep born in 1994 or earlier (therefore four to six year old when tested) strengthens the assumption that the Merino sheep introduced from Farm X did not shed sufficient numbers of MAP prior to 1994 to contaminate the environment and transmit infection to lambs present on the study farm at that time.

Whole flock testing using PFC of all adult sheep in 2000 confirmed that shedding was limited to the 1995-born age group. It is possible that contamination originating from the Merino sheep was very localized and only the 1995-born commercial Bond ewes were exposed (i.e. by chance). Alternatively, and more likely considering subsequent test results, age susceptibility to infection meant that only lambs exposed to contamination developed a patent infection. If this is the case and if the Merino sheep produced sufficient contamination with MAP from 1995 and lasting until 12 months after their departure from the study farm in 1996, then sheep born in 1995, 1996 and 1997 were at risk of infection, but only the 1995-born sheep were old enough to shed at the time of sampling in 2000.

Results from testing in 2001 and 2002 support this hypothesis, and showed that in 2001 shedding was occurring in the 1996-born sheep and in 2002 in 1997-born sheep. Contamination left by the Merino sheep was probably quite low, leading to lower ingested doses of MAP, which would explain the late onset of shedding (5 year old) in the 1995-, 1996- and 1997-born age groups (Whittington et al, 2001). Also these sheep were in good body condition and perhaps other environmental or genetic effects contributed to late onset of shedding. It is also possible that the 1996- and 1997-born sheep became infected as adults from contamination by homebred Bond ewes that were confirmed to shed at the 2000 and 2001 testing (Fig.1). However we consider this unlikely due to insufficient time for development of lesions and shedding and the low prevalence of shedding (Table 1).

At the 2001 testing, shedding was confirmed in the 1999-born stud ewes but not in the commercial ewes of the same age (Table 1). The source of infection of this age group is uncertain. Infection could have been transmitted by chance exposure to high contamination (produced by homebred sheep shedding in 2000, 2001 or earlier). This is unlikely because the prevalence in homebred sheep was low (see table 1). Intrauterine infection may have been the source as
their dams could have been the 1995- or 1996-born infected ewes. However intrauterine transmission is improbable unless sheep are clinically infected, and there were no clinically infected sheep observed on the study farm (Lambeth et al, 2004). The same mob, when retested in 2002 was apparently no longer shedding bacteria. Intermittent shedding may reflect either stage of disease (Whittington et al, 2001), passive shedding of ingested organisms acquired from contaminated pasture or perhaps the effect of vaccination (Eppleston et al, 2003). Alternatively, the sheep being responsible for shedding could have died during the interval between the two tests.

**Table 1.** The prevalence of OJD infection based on AGID and PFC test results, for groups of samples where either test was positive

<table>
<thead>
<tr>
<th>Test date</th>
<th>Year of birth</th>
<th>Sample size</th>
<th>Breed</th>
<th>No. sheep/pool</th>
<th>Test</th>
<th>No. pos.</th>
<th>Prevalence of sero-positive or faecal culture positive sheep % (95% C.L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct 1997</td>
<td>1990/91</td>
<td>239</td>
<td>Merino</td>
<td>na</td>
<td>AGID</td>
<td>9b</td>
<td>3.8 (1.7 – 7.0)</td>
</tr>
<tr>
<td>Mar 2000</td>
<td>1995</td>
<td>95</td>
<td>Bond</td>
<td>na</td>
<td>AGID</td>
<td>4b</td>
<td>4.2 (1.7 - 11.9)</td>
</tr>
<tr>
<td>Jun 2000</td>
<td>1995</td>
<td>202 (10 pools)</td>
<td>Bond</td>
<td>20-22</td>
<td>PFC</td>
<td>4</td>
<td>2.5 (0.1 - 5.0)</td>
</tr>
<tr>
<td>Sep 2001</td>
<td>1999</td>
<td>100 (2 pools)</td>
<td>Bond</td>
<td>50</td>
<td>PFC</td>
<td>1</td>
<td>1.4 ( 0 - 4.1)</td>
</tr>
<tr>
<td>Oct 2001</td>
<td>1996</td>
<td>306 (6 pools)</td>
<td>Bond</td>
<td>50-56</td>
<td>PFC</td>
<td>2</td>
<td>0.8 ( 0 - 1.9)</td>
</tr>
<tr>
<td>Oct 2002</td>
<td>1996</td>
<td>350 (7 pools)</td>
<td>Bond</td>
<td>50</td>
<td>PFC</td>
<td>5</td>
<td>2.5 (0.2 – 4.8)</td>
</tr>
<tr>
<td>Oct 2002</td>
<td>1997</td>
<td>325 (7 pools)</td>
<td>Bond</td>
<td>26(1 pool), 49-50</td>
<td>PFC</td>
<td>1</td>
<td>0.3 ( 0 - 0.9)</td>
</tr>
</tbody>
</table>

AGID: Agar gel immunodiffusion; PFC: Pooled faecal culture; a not applicable; all sheep were tested individually; b number of positive individuals

**CONCLUSION**

The history and extensive testing available on this farm allowed identification of the time period over which mycobacterial contamination occurred by the introduced infected Merino sheep. The study highlights the slow spread of infection within the home bred flock. OJD infection was clustered, remaining limited to one or two age groups for at least seven years after the infection was introduced.

Opportunities for infection control and risks to effective surveillance are apparent retrospectively. On this farm, it may have been possible to stop transmission of OJD through timely implementation of infection management practices such as selective culling, strategic grazing and vaccination. If these disease control strategies had been implemented earlier than was possible on this farm, transmission may have been stopped.

In retrospect, action that could have been taken in 1996/97 that may have led to elimination of the infection from the study farm would include:

- Implementation of an infected flock profile immediately upon identification of the infected introduced sheep
- Culling of these sheep and their progeny
- Culling of home bred sheep born during the preceding and following 12 months, corresponding to the period of contamination, and prior to reaching 12 months of age
- Implementation of a vaccination program of all remaining sheep and annually of lambs at marking time
- Whole flock testing using PFC (infected flock profile) about 5 years after commencement of the program to monitor flock infection status

Vaccination was used on the study farm and the owner plans to vaccinate ewe lambs annually at marking or weaning. Based on recent research results with Gudair ® vaccine under Australian conditions, vaccination on this farm is likely reduce the levels of MAP contamination as well as delay the onset of shedding (Eppleston et al, 2003). It may be that in very low challenge situations such as this, vaccination eventually prevents shedding of detectable levels of MAP. Further testing would be needed to confirm this.
This study demonstrates how difficult effective OJD surveillance may be unless exact history is known and the correct age groups or mobs are tested or unless testing occurs over a long period of time. Clustering of infection reduces the confidence in a negative test outcome if sampling is random. This has significant implications for market assurance program testing (Whittington et al. 2001) and surveillance testing undertaken to assess the OJD risk of a flock or mob of sheep intended for sale.

Fig. 1. Representation of MAP spread on the study farm. Sheep mobs are indicated by horizontal bars where the left edge of each bar indicates when sheep were born and the right edge when sheep were tested. Dark bars indicate mobs with positive results in tests for OJD, while white bars indicate mobs that had negative test results. The dark block represents the likely interval of environmental MAP contamination from introduced merino sheep, while light dotted block represents the likely period of environmental MAP contamination from homebred Bond sheep.
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