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Ovine strain of *Mycobacterium paratuberculosis* in beef cattle: A case study

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Abstract

A single beef cattle herd in the Ballarat region of Victoria was found to be infected with ovine (S) strain *Mycobacterium avium* subspecies *paratuberculosis* in May 2005. The property had a history of clinical Johne's disease in sheep during the period 1998 to 2002. The owner of the herd elected to remove (for slaughter) all the cattle on the property. This decision provided a unique opportunity to obtain blood, faecal and tissue samples from all the cattle, at or before, the time they were slaughtered. Samples were processed and examined for evidence of *M paratuberculosis* and for S strain in particular.

Laboratory results indicate that S strain infection was more widespread in the cattle herd than was expected from the clinical presentation of the disease, the results of initial serological testing or ante-mortem faecal culture results.

The significance of these findings on Johne's disease control programs for cattle and sheep are discussed. In particular, it is suggested that herd grazing/feeding strategies that reduce the risk of transmission of S strain *M paratuberculosis* from infected sheep to cattle should be adopted.

Executive summary

Johne's disease is a chronic granulomatous enteritis of ruminants and camelids that is caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Mptb)*. In cattle, the disease typically presents as intractable diarrhoea and severe wasting in mature animals.

The observation that cattle do not become clinically affected by Johne's disease when grazing with infected sheep for extended periods and the fact that culture media required for laboratory growth of isolates from sheep or cattle strains are different, support the hypothesis that there are distinct "strains" of *Mptb;* a cattle (C) strain that predominantly affects cattle but is also capable of infecting goats, deer and alpaca, and a sheep (S) strain that predominantly affects sheep. Recent Australian studies[2,3,4] using polymorphism in the IS1311 element and other DNA techniques have confirmed this general proposition.

However, it is now clear that this species-strain adaptation or preference is not absolute. S strain *Mptb* was retrospectively diagnosed from archived histological sections collected in the late 1990s from three individual cattle from three NSW properties[3] and investigation of clinical Johne's disease cases in Victorian cattle has identified further cases of cross-infection.

Detection of S strain *Mptb* in cattle raises the possibility that control of Johne's disease using current strategies which assume there is no cross-infection between cattle and sheep could, on occasions, be compromised.

This project was undertaken to determine the within herd distribution of S strain *Mptb* infection in cattle in a single beef cattle herd in the Ballarat region of Victoria, to provide a better understanding of the epidemiology of S strain *Mptb* infection in cattle and assist in the development of control strategies for other similarly affected herds.

Blood, faecal and tissue samples from all the cattle, at or before, the time they were slaughtered were obtained and samples were processed and examined for evidence of *Mptb* and for S strain in particular. Herd management information was collected to enable a profile of the Johne's disease situation within the herd to be developed. This included purchase records, grazing history, calving records, age, sex and exposure to infected sheep or sheep faeces as calves.

In total, 73 head of cattle were sampled before and after slaughter. Fifteen animals (20.5% of the herd) returned positive results to at least one of the non-serological tests for *Mptb* infection applied in this herd and 14 animals (19.2% of the entire herd) returned positive results to at least one of the definitive, post-mortem (histology or tissue culture) tests for *Mptb* infection applied. The positive animals ranged from in age from15 months to 6 years.

Initial (ante-mortem) testing in the herd using ELISA and faecal culture suggested that infection with S strain *Mptb* in the study herd was confined to cows in the 5 and 6-year old age groups. Further investigation including thorough post-mortem examination and testing revealed that infection was widespread throughout the herd. All infected cattle from which organisms could be cultured were shown to be infected with S strain *Mptb*.

The sensitivity of each of the tests employed in this study was determined by comparing the number of animals detected by the test with the total known to be infected. No combination of ante-mortem tests provided a test regime with more than 36% sensitivity.

In addition to determining the prevalence of disease in the study herd we sought to examine the relationship between possible risk factors for disease transmission to cattle from sheep. Co-grazing of cattle (in particular calves) and ovine Johne's disease (OJD) infected sheep, direct exposure of cattle to infected pastures (cattle following infected sheep) and indirect exposure of cattle to infected pastures (cattle grazing paddocks contaminated by run-off from OJD infected neighbouring flocks) were all occurring on the study property from at least 2002. It is likely that increasing severity (number of clinical cases or high mortality), prevalence and duration of OJD infection in the resident and neighbouring flocks increases the likelihood of cattle acquiring S strain infection.

Other factors such as drought and hand feeding sheep and cattle (leading to grazing close to the ground for both cattle and sheep) are thought to increase *Mptb* transmission to cattle. These factors were present in the recent history of the study herd.

This study has implications for the current Johne's disease control and accreditation programs in south eastern Australia. Historically, it has been assumed that cattle do not become clinically affected by Johne's disease when grazing with *Mptb* infected sheep for extended periods and that cattle to cattle transmission of S strain *Mptb* does not occur. Many properties undertaking OJD control programs run cattle as an additional or alternative enterprise. The owners and managers of OJD infected flocks (and their advisors) should consider the risk factors that create favourable circumstances for cross species transmission of *Mptb*. Specifically, the results of this study support recommendations that

- When cattle are being reared in OJD endemic areas care should be taken to ensure that they are not exposed directly or indirectly to infected sheep or manure (including contaminated run-off from neighbouring properties) until the cattle are at least 12 months old.
- Care should be taken when hand feeding cattle to avoid areas of high sheep manure build up.
- Diagnostic testing of suspect clinical cases should routinely involve strain typing of any cultivated *Mptb*.
- Disease control and accreditation programs for both OJD and for bovine Johne's disease should include consideration of the possibility that S strain Mptb may be transmitted to cattle

Although S strain *Mptb* infection of cattle is currently a sporadic and relatively rare event, changes to the current use of alternative species (cattle) for pasture decontamination of S strain *Mptb* are warranted.

Study conclusions;

- Diagnostic testing of suspect clinical cases of bovine Johne's disease should routinely involve strain typing of any cultivated *Mptb*.
- Existing diagnostic tests may be used to detect cattle infected with S strain Mptb. Strain typing is required to distinguish C and S strain infections.
- This study provided the first documented estimates of the sensitivity of diagnostic tests for S strain *Mptb* infection in cattle.
- Ante-mortem tests (ELISA and faecal culture) have poor sensitivity except when applied to cattle in the latter stages of disease pathogenesis (5 years old and older).
- The true prevalence and distribution of S strain Mptb infection in a herd is therefore difficult to estimate using existing ante-mortem diagnostic tests.
- The on-going source of infection of cattle in this study remains unclear but could include infected and shedding adult cattle, infected and shedding sheep resident on the study property or organisms in the environment originating from contaminated water draining from neighbouring OJD affected properties.
- Disease control and accreditation programs for both OJD and for bovine Johne's disease should include consideration of the possibility that S strain Mptb may be transmitted to cattle
- Taking the most cautious approach, only adult cattle should be grazed on areas recently grazed by known OJD infected sheep or contaminated by run-off from adjacent land holdings that may have grazed OJD infected sheep.

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1 Background

Johne's disease is a chronic granulomatous enteritis of ruminants and camelids that is caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Mptb)*. In cattle, the disease typically presents as intractable diarrhoea and severe wasting in mature animals. The route of transmission is largely faecal-oral, with very large numbers of organisms being shed in the faeces of clinically affected animals. Fewer, but still significant numbers of organisms are shed by clinically-normal-infected animals.[1]

The observation that cattle do not become clinically affected by Johne's disease when grazing with infected sheep for extended periods and the fact that culture media required for laboratory growth of isolates from sheep or cattle are different, support the hypothesis that there are distinct "strains" of *Mptb;* a cattle (C) strain that predominantly affects cattle but is also capable of infecting goats, deer and alpaca, and a sheep (S) strain that predominantly affects sheep. Recent Australian studies[2,3,4] using polymorphism in the IS1311 element and other DNA techniques have confirmed this general proposition.

However, it is now clear that this species-strain adaptation or preference is not absolute. S strain *Mptb* was retrospectively diagnosed from archived histological sections collected in the late 1990s from three individual cattle from three NSW properties[3]. The cases occurred between 1989 and 1995 on properties in the central tablelands of NSW, an area where ovine Johne's disease was and is, endemic. Maloney and Whittington[5] reported the results of opportunistic investigations on two NSW farms undertaking the Australian Johne's Disease Market Assurance Program for Cattle (CattleMAP). Whole herd ELISA testing revealed two test positive animals in each herd that were subsequently shown to be infected with S strain *Mptb*. Only one of the three cattle showed clinical signs of Johne's disease.

Investigation of clinical Johne's disease in Victorian cattle has identified further cases of cross-infection. In 1999, a 155 cow dairy/beef herd that had grazed with sheep in which ovine Johne's disease had been diagnosed was found to contain at least five cattle identified as histologically positive or positive on faecal or tissue culture for S strain *Mptb*. The sheep flock was removed from the property in 1999 and the last confirmed bovine case of S strain *Mptb* occurred in the herd in June 2002.

More recently, cattle in two beef herds in the Ballarat region have been found to be infected with S strain *Mptb.* One property had a history of relatively severe ovine Johne's disease in sheep in the period 1998 to 2002; the other had no history of ovine Johne's disease and no clear external source of infection.

Detection of S strain *Mptb* in cattle raises the possibility that control of Johne's disease using current strategies which assume there is no cross-infection between cattle and sheep could, on occasions, be compromised.

This project was undertaken to determine the within herd distribution of S strain *Mptb* infection in cattle in one of the Ballarat herds, to provide a better understanding of the epidemiology of S strain *Mptb* infection in cattle and assist in the development of control strategies for other similarly affected herds.

2 Project objectives

To report the findings of a detailed investigation in a beef herd infected with S-strain *Mycobacterium avium* subspecies *paratuberculosis* (*Mptb)*

Specifically to:

- Describe in detail the history of infection and management of the resident sheep flock
- Describe in detail the history of infection and management of the beef herd
- Describe the within herd distribution and prevalence of infection with S strain Mptb in different cohorts (age/sex/origin/lineage) within the herd.
- Provide a better understanding of the epidemiology of S strain Mptb infection in cattle and assist in the development of control strategies for other similarly affected herds.
- Attempt to identify likely risk factors for infection of the herd and cohorts within the herd.
- Attempt to determine if S-strain Mptb has been transmitted between cattle within the herd.

3 Methodology

3.1.1 Study Design

A cross-sectional design was employed in this study. The owner of the affected herd elected to remove (for slaughter) all the cattle on the property. Blood, faecal and tissue samples were collected from all animals in the herd at or before, the time the animals were slaughtered. Samples were processed and examined for antibody, histological or cultural evidence of *Mptb* infection, in particular presence of S strain *Mptb*.

Herd management information was collected to enable a profile of the Johne's disease situation within the herd to be developed. This included purchase records, grazing history, calving records, age, sex and exposure to infected sheep or sheep faeces as calves.

3.1.2 Sample collection and processing for serological testing

Blood samples were collected from the tail vein using plain vacutainer tubes. Samples were allowed to clot at room temperature and then packaged for transport to Gribbles Veterinary Pathology. On arrival the serum was tested using the bovine Johne's disease absorbed enzyme-linked immunosorbent assay (ELISA) for antibodies against *Mptb*. The agar gel immunodiffusion (AGID) test was also performed on serum[6].

3.1.3 Sample collection and processing for faecal culture

Faeces were collected from the rectum of each animal in 70ml sterile containers at the same time as the collection of blood and prior to despatch of the animal to the abattoir. A new glove was used for each animal to prevent cross contamination. Samples were chilled and sent to Gribbles Veterinary Pathology within 12-24 hours of collection.
Upon receipt at Gribbles Pathology samples were subjected to the following five processing steps[6]

1. Decontamination of the specimen in hexadecylpyridinium chloride (HPC).
2. Liquid media culture using a BACTEC technique where growth in liquid medium is identified by the detection of radio labelled metabolites enabling isolation of either or both S and C strains of *Mptb*.
3. PCR on liquid media from step 2, using a polymerase chain reaction (PCR) targeting the insertion sequence IS*900* of *Mptb* followed by typing of isolates as cattle 'C' or sheep 'S' strain by amplification and restriction of the IS*1311* gene. Strain typing was performed on all positive faecal or tissue cultures for both strains.
4. Solid media culture to confirm mycobactin dependency and differentiate *Mptb* from other Mycobacteria.
5. Ziehl-Neelsen (ZN) staining of colonies of suspected *Mptb* to identify acid-fast bacilli .

When there was no growth in liquid (BACTEC) medium the result was reported as negative.

When growth was detected in liquid (BACTEC) medium, further testing involving PCR, ZN staining and attempted cultivation on solid media were undertaken to determine whether the growth was caused by *Mptb* and to determine the strain as either cattle 'C' or sheep 'S' strain.

3.1.4 Slaughter and post-mortem examination

All cattle in the herd were slaughtered at abattoirs in four consignments between the 1st of March 2006 and the 23rd of November 2006. Each was examined by the project manager or assisting DPI Animal Health Officers and District Veterinary Officers. A standard set of 7 sites were sampled as outlined in the Australian Cattle MAP guidelines[7].

Two sets of tissues were collected including ileocaecal lymph node, ileaocaecal valve, distal ileum, ileum, mesenteric lymph node, proximal colon and caecum.

Significant findings on gross examination such as thickened ileum, enlarged lymph nodes or caecal beading were noted at the time of sampling.

3.1.5 Sample collection and processing for histopathology

Once collected, one set of tissues were fixed in 10% buffered formalin and sent to Gribbles Veterinary Pathology for processing and examination. Samples were embedded in paraffin, stained for normal tissue elements with haemtoxylin and eosin and also for acid fast bacilli using the Ziehl Neelsen method[6].

3.1.6 Sample collection and processing for bacteriological culture of tissue

The other set of tissues remained un-fixed and were submitted to Gribbles Veterinary Pathology for processing and examination. Bacteriological culture was performed using the same steps as described in 3.1.3 for faecal culture.

3.1.7 Herd management data collection

Face to face interviews were conducted with the herd owner and herd manager and relevant files held in the local and regional DPI offices were reviewed to obtain herd and flock management information and details of the Johne's disease history in the herd and flock.

4 Results

4.1.1 Testing outcomes

In total, 73 head of cattle were sampled before and after slaughter.

The herd demographics and the outcomes from testing are shown in Tables 1 and 2. In summary, all of the cattle returned negative AGID blood tests for ovine Johne's disease. Four, 6-year old cows returned positive ELISA results. Three of these cows also returned positive faecal cultures that were identified as S strain *Mptb* using PCR. A 5-year old animal was found to be histologically positive but was ELISA negative. Another 5-year old cow was faecal culture positive but was not positive on histological or tissue culture examination.

Ten animals (two 15 month-old heifers, one 15 month-old steer, one 5 year-old and six 6 year-old cows) were positive for Johne's disease on histopathological examination of tissue sections however pathology reports indicate that only small numbers of acid-fast bacilli were detected in eight of the animals while one cow had moderate numbers of acid-fast bacilli. An additional 6 year-old cow showed lesions suggestive of Johne's disease with a few small clumps of epithelioid macrophages and giant cells in the cortical area; however no acid fast bacilli were visible in these lesions.

Mptb was grown from gut or lymph-node tissue samples from eleven animals (two 15 month-old heifers, one 15 month-old steer, one 2 year-old heifer, two 3 year-old cows and five 6 year-old cows). Ovine strain was confirmed in all samples. No C strain infection was detected in the herd.

In total, 15 animals (20.5% of the herd) returned positive results to at least one of the non-serological tests for *Mptb* infection applied in this herd and 14 animals (19.2% of the entire herd) returned positive results to at least one of the definitive, post-mortem (histology and tissue culture) tests for *Mptb* infection applied in this herd. The positive animals ranged from in age from14 months to 6 years and all but one was female.

4.1.2 Diagnostic test sensitivity

Given that all the cattle in the herd were slaughtered and subjected to the same thorough post-mortem examination, the true prevalence of S strain *Mptb* infection in the herd (defined as the proportion of the herd that is disease positive as determined by any combination of definitive tests (histological positive and/or tissue culture positive)[8] was found to be 19.2%. The sensitivity of each of the tests employed in this study was determined by comparing the number of animals detected by the test with the total known to be infected (Table 3). No combination of ante-mortem tests provided a test regime with more than 36% sensitivity.

Test specificity could not be determined from the study data[8].

4.1.3 Property location and description

The study property comprised 400 hectares in the Ballarat region and ran both sheep and cattle. A number of paddocks were grazed by cattle and sheep, and some by sheep only. The soil type is predominantly grey basalt and the pH has been measured at 4.8 and 6.0 in each of the paddocks. There are 3 paddocks that have a proportion of red volcanic soil also. Major pasture species are rye and clover which is rotationally grazed. There is one paddock of lucerne that is grazed by sheep only.

Water is provided to stock by a spring fed creek running with the exception of summer months, through three paddocks, (sheep and cattle). A large dam (in a sheep paddock) provides water which is pumped to various troughs across the property.

4.1.4 History of infection and management of Johne's disease in sheep on the property

Diagnosis of Johne's disease
Ovine Johne's disease (OJD) was diagnosed on the property in August 2003 following an abattoir trace of three infected sheep from a line of 1063 processed in February 2003. At the time the abattoir inspector noted that over half of the consignment appeared to be affected.

In 2003 the flock comprised 2886 head, the majority were ewes aged between 2 and 4 years. The manager reported that he had experienced several years of unexplained deaths in the sheep and thus it is likely that OJD had been present in the flock for a number of years prior to the initial diagnosis. Mortality rates prior to August 2003 are estimated at around 15-18 % of the flock. In one reported incident the manager picked up 72 dead sheep in one day.

A further four positive abattoir traces were detected after the initial diagnosis of OJD (January 2004 to November 2006)

Testing
Post mortem examinations on four sheep (2 ewes and 2 wethers) were carried out on the property in August 2003 to establish the flock OJD status following the index

abattoir trace. All four sheep submitted for post mortem were found positive on histopathology and AGID blood test. Further post mortem examinations were conducted in September 2003 and January 2004 all were positive on AGID and histopathology.

A full flock pooled faecal culture (PFC)[9] was conducted in October 2003 to determine the prevalence of *Mptb* infection in the flock. Three hundred and fifty animals were tested, 7 pools were submitted each returning a positive faecal culture result.

Control
Due to the high level of clinical losses, 500 ewes considered to be a high risk for *Mptb* infection were culled in January and February 2004.

Johne's disease vaccination of lambs using "Gudair" vaccine[10,11] began in 2003. All sheep on this property at the date of writing are now vaccinated.

Since diagnosis and implementation of the vaccination program, mortality in the flock has dropped significantly. In the first year of vaccination the manager reported an 8 to10 % drop in mortality.

Currently, after 5 years of vaccination, the mortality rate on this property is 2% of the 2,500 head

Three of the four neighbours surrounding the property were diagnosed with high prevalance OJD in the period 2003-2004 and the fouth is suspected of being infected but the condition has (to date) not been confirned. Drainage from infected neighbours flows into the study property.

Flock management
The resident sheep flock is a self replacing merino flock with the only purchase being rams from an Australian Johne's Disease Market Assurance Program for Sheep (SheepMAP) Monitered Negative level 3[9] stud flock, annually in October.

Sheep are grazed across the entire property, in all paddocks and up to a density of 10 DSE/ha. Grazing was undertaken concurrently with cattle in many instances.

4.1.5 History of S strain Mycobacterium paratuberculoisis infection in beef cattle on the property

Diagnosis of Johne's disease
The first case of Johne's disease in cattle on the property was diagnosed in May 2005. The five year old cow was scouring and exhibited weight loss that was non-responsive to treatment for intestinal worms and to the provision of supplementary feed. She was clinically unwell for several weeks before being referred for veterinary attention. At slaughter the animal was ELISA positive and returned positive results for bacteriological culture on intestinal tissue. Further examination (which is a routine procedure for all *Mptb* positive cultures in Victoria (Pers comm Tristan Jubb) resulted in the detection of S strain *Mptb* in the tissues.

Subsequent investigation on the property revealed that this was the third bovine animal to show similar clinical signs. The first died in December 2004 and was buried on property. The second began to deteriorate and was sold for slaughter in March 2005.

Herd management
The original Shorthorn herd had been in existence for more than 20 years. An Angus bull had been crossed over the base Shorthorn herd from the early 1990s. The herd was self replacing, purchasing bulls only. No cattle agistment has been undertaken on the property.

There had been no significant cattle health issues identified in the herd with the exception of selenium deficiency which was diagnosed in 2000 and treated on one occasion with slow release bolus capsules.

Calving occurred in July, with calves weaned 9 to 10 months later. Steer calves were sent for sale then slaughter via Ballarat cattle market. Heifer calves were weaned in the cattle yards and hand fed for a week before being moved.

Hand feeding of the herd was undertaken between February and September with cattle being fed lucerne hay and/or grass hay from the property.

Exposure of calves/weaners to sheep
Four paddocks were co-grazed by sheep and cattle. Lambing occurred in three of these four paddocks while weaner sheep ran with weaner cattle in the fourth paddock.

All calves were exposed to drainage from neighbours with OJD.

All ages and classes of cattle consistently grazed concurrently with sheep including on occassions, sheep with clinical signs of OJD.

Table 1. Results of testing 73 animals from a beef herd in the Ballarat district know to be infected with S strain *Mycobacterium paratuberculosis* using AGID ELISA, faecal culture, histopathology and bacteriological culture of tissue following slaughter.
* Infection with *Mptb* was deemed to be confirmed if tissue samples collected at slaughter were found to positive on histological examiniation or on bacteiological culture.

Animal details		Number tested	AGID positive	ELISA positive	Faecal culture positive	Histologically positive	Tissue culture positive	Number confirmed infected* (% of age group)
Sex	**Age (rearing year)**							
Bull	3-4 yo (N/A - purchased)	4	0	0	0	0	0	0 (0%)
Steers	15 mth (2005)	11	0	0	**0**	1	1	1 (9%)
Heifers	15 mth (2005)	18	0	0	**0**	2	2	3 (17%)
Heifers	2 yo (2004)	10	0	0	**0**	0	1	1 (10%)
Cows	3 yo (2003)	2	0	0	**0**	0	2	2 (100%)
Cows	4 yo (2002)	7	0	0	**0**	0	0	0 (0%)
Cows	5 yo (2001)	10	0	0	**1**	1	0	1 (10%)
Cows	6 yo (2000)	11	0	4	**3**	6 (plus 1 suggestive?)	5	6 (55%)
Total (% of herd)		**73**	**0 (0%)**	**4 (5.5%)**	**4 (5.5%)**	**10 (14%)**	**11 (15%)**	**14 (19.2%)**

Table 2. Results of testing 73 animals from a beef herd in the Ballarat region known to be infected with S strain *Mycobacterium paratuberculosis* using ELISA, faecal culture and histopathology and bacteriological culture of tissues following slaughter – agreement between tests.

Animal details		ELISA result	Faecal culture result	Result of histological examination	Tissue Culture result	Disease status
Sex	**Age**					
Steer	**15 mths**				+	+
Heifer	**15 mths**				+	+
Heifer	**15 mths**			+		+
Heifer	**15 mths**			+	+	+
Heifer	**2 yo**				+	+
Cow	**3 yo**				+	+
Cow	**3 yo**				+	+
Cow	**5 yo**			+		+
Cow	**5 yo**		+			?
Cow	**6 yo**	+	+	+	+	+
Cow	**6 yo**	+	+	+	+	+
Cow	**6 yo**	+	+	+	+	+
Cow	**6 yo**			+	+	+
Cow	**6 yo**			+		+
Cow	**6 yo**	+		+	+	+
Cow	**6 yo**			? (suggestive)		?

Table 3. Sensitivity of diganostic tests (AGID, ELISA, faecal culture, histopathology and bacteriological culture of tissues following slaughter)when applied to 73 animals from a beef herd in the Ballarat region known to be infected with S strain *Mycobacterium paratuberculosis (Mptb)*.
* Infection with *Mptb* was deemed to be confirmed if tissue samples collected at slaughter were found to positive on histological examiniation or on bacteiological culture.

Diagnostic test	Total number of confirmed *Mptb* infected cattle*	Number of test cattle positive	Test sensitivity	95% Confidence interval
AGID	14	0	0	0
ELISA	14	4	28.6%	5% - 52%
Faecal culture	14	4	28.6%	5% - 52%
ELISA and faecal culture in parrallel	14	5	35.7%	10.6% - 60.8%
Histopathology	14	10	71.4%	47.8% - 95.1%
Tissue Culture	14	11	78.6%	57.1% - 100%

5 Discussion

Initial (ante-mortem) testing in the herd using ELISA and faecal culture suggested that infection with S strain *Mptb* in the study herd was confined to cows in the 5 and 6-year old age groups. Further investigation including thorough post-mortem examination and testing revealed that infection was widespread throughout the herd including three 15-month old animals (two heifers and a steer). In total, 14 animals (19.2% of the entire herd) in five age groups returned positive results to at least one of the definitive tests for *Mptb* infection applied in this herd. All infected cattle from which organisms could be cultured were shown to be infected with S strain *Mptb*.

One five year old cow was found to be faecal culture positive but was negative when examined histologically and with bacteriological culture following slaughter. Other studies[5] have been ambivalent about the disease status of such cattle. On one hand they suggest that the animal may be not infected but acting as a passive shedder, mechanically transmitting ingested organisms. On the other they suggest that the animal may be truly infected and could be used to calculate true disease prevalence in the herd/cohort of origin. Certainly, in a high challenge environment such as the current study herd it may be possible for an animal to be a passive shedder of detectable organisms. Equally, it is possible that she may be infected but the post-mortem sample collected failed to include diseased tissue. The authors do not exclude the possibility that the animal was infected, but to allow the most rigorous assessment of the herd situation and test performance our disease prevalence and test sensitivity estimates have been based on figures calculated after excluding the results from this individual.

It is of interest that four animals less than two years of age in 2006 were found to be infected with *Mptb.* This group of animals should have been raised in an environment of decreasing challenge (as there were fewer clinically infected sheep) and should have had the lowest prevalence of *Mptb* infection. In this study 14% (4/29) 15-month old animals were detected as infected with *Mptb*. Given that pathological changes were likely to be minimal in such young animals even if they were infected, it seems probable that more young animals were infected but remained undetected and that the real prevalence of infection in this age group was even higher than our estimates suggest (and test sensitivity correspondingly lower than the estimate calculated).

The source of infection in the young cattle remains unclear. Unfortunately, records of cow/calf relationships were not kept for this herd as these might have allowed an investigation of possible vertical transmission of S strain *Mtpb* from dam to calf. Another possible source of infection for these animals is contact with other infected older cows in close proximity. Certainly the calves would have had considerable contact with many infected animals as they were being reared and up to weaning. We have demonstrated that S strain *Mptb* may be readily cultivated from the faeces of infected cattle. Consequently, it seems likely that these cattle may be a source of infection to others in the herd. The age at which this susceptibility ceases (if indeed it does) cannot be established from the current work in a single herd.

Alternatively, the only other source of infection for the calves is direct or indirect contact with infected sheep. If this were the case it would suggest that there is still considerable environmental contamination of the calving and calf rearing areas by sheep on the property or from contaminated drainage from neighbouring properties. No recent assessment has been made of the overall prevalence of *Mptb* in the resident sheep flock or neighbouring flocks or of the prevalence of vaccinated sheep shedding *Mptb* organisms while remaining visibly healthy, but these measures would

be of significance in determining the most likely source of on-going infection in the study herd.

Only the 4-year old cow cohort (7 in total) and the purchased bull group were found to contain no test positive animals. It is likely that this is an accurate reflection of the infection status of the bulls since they were purchased as rising 2-year old animals and unlikely to have been infected with S strain *Mptb* on their properties of origin. It is less clear that the 4-year old cows were free of *Mptb* infection. The diagnostic tests may simply have failed to detect an infected cow in this group, although given the apparent success in detecting infection in all other age groups this would seem unlikely. We were unable to establish any differences in management procedures that occurred around 2002 (when these cows were being born and reared) that might explain the lack of infection in the 4-year old cow age group. In fact, this group were the cohort arguably most exposed to challenge from infected sheep since this was the year immediately prior to the diagnosis of OJD in the sheep flock when clinical disease and mortality from OJD in the sheep was at its peak.

This study has provided the first documented estimates of the sensitivity of diagnostic tests for S strain *Mptb* infection in cattle. In common with diagnostic tests for Johne's disease generally ante-mortem tests (ELISA and faecal culture) appear to have poor sensitivity except when applied to cattle in the last stages of disease pathogenesis (5 years old and older). As a result of these findings we suggest that more extensive and invasive testing (including the use of mesenteric lymph node biopsy) should be considered before excluding disease in cattle under 4 years of age. Alternatively, an apparently low prevalence of *Mptb* infection as measured by ELISA or faecal culture, or disease detected only in older animals should NOT be taken as an indicator of overall low disease prevalence in the herd.

In addition to determining the prevalence of disease in the study herd we sought to examine the relationship between possible risk factors for disease transmission to cattle from sheep. Co-grazing of cattle (in particular calves) and OJD infected sheep, direct exposure of cattle to infected pastures (cattle following infected sheep) and indirect exposure of cattle to infected pastures (cattle grazing paddocks contaminated by run-off from OJD infected neighbouring flocks) were all occurring on the study property from at least 2002. Consequently it is impossible for us to separate the effect of any one of these factors from the others or to prioritise them. Clearly it is likely that increasing severity (number of clinical cases or high mortality), prevalence and duration of OJD infection in the resident and neighbouring flocks increases the likelihood of cattle acquiring infection.

Other factors such as drought and hand feeding sheep and cattle (leading to grazing close to the ground for both cattle and sheep) are thought to increase *Mptb* transmission to cattle. These factors were present in the recent history of the study herd.

The results of this study may be the consequence of studying an unusual, arguably even rare, event; however they challenge a number of current assumptions about S strain *Mptb* infection in cattle.

- It is generally assumed that cattle are not readily infected with S strain *Mptb*. The finding that at least 19.2% of the herd are infected suggests that given favourable circumstances cattle may be readily infected but may not be **detected** as infected unless post-mortem testing is undertaken.
- It is generally assumed that infection does not progress to clinical disease in cattle. The herd under examination in this study experienced one confirmed clinical case and two other probable clinical cases in under 12 months.

- Finally, it is assumed that cattle to cattle transmission of S strain *Mptb* does not occur. The high prevalence of infection and evidence for on-going transmission within, or to, the cattle herd despite apparent control in the resident sheep flock suggests that cattle to cattle transmission should be considered possible if not probable.

This study was unable to investigate other features of the epidemiology of S strain *Mptb* infection in cattle (ie, whether age related susceptibility exists with calves most susceptible and adult cattle resistant to infection; and the possibility of transmission of the S strain organism *in-utero* and via milk or colostrum). Larger, longitudinal studies are required to investigate these interesting issues.

The most significant impact from this work will be the implications for the current Johne's disease control and accreditation programs in south eastern Australia. Historically, it has been assumed that cattle do not become clinically affected by Johne's disease when grazing with *Mptb* infected sheep for extended periods and that cattle to cattle transmission of S strain *Mptb* does not occur. Many properties undertaking OJD control programs run cattle as an additional or alternative enterprise.

The conclusions from this study have implications for the current Johne's disease control programs in south eastern Australia and support the recommendations made by Maloney and Whittington[5] who argued that

- When cattle are being reared in OJD endemic areas care should be taken to ensure that they are not exposed directly or indirectly to infected sheep or manure (including contaminated run-off from neighbouring properties) until the cattle are at least 12 months old.
- Care should be taken when hand feeding cattle to avoid areas of high sheep manure build up.
- Diagnostic testing of suspect clinical cases should routinely involve strain typing of any cultivated *Mptb*.
- Disease control and accreditation programs for both OJD and for bovine Johne's disease should include consideration of the possibility that S strain Mptb may be transmitted to cattle.

Although S strain *Mptb* infection is a sporadic and relatively rare event, changes to the current use of alternative species for pasture decontamination are warranted.

Finally, this study may provide an insight into one possible future for Johne's disease epidemiology in Victoria. Until OJD became endemic in the sheep flock in this State, Johne's disease as a clinical entity was largely restricted to the dairy industry. Since 1995, the exposure of the "pure" beef industry generally to *Mptb* has increased significantly. Even if only small percentage of the 20,000 herds in Victoria are exposed to sufficient Mptb challenge to allow disease transmission to the cattle it is likely that the prevalence of Johne's disease in beef herds will increase and that the relative frequency of infection with S strain *Mptb* will increase. Active management to protect the beef industry now may prevent many new cases of Johne's disease and reduce the potential for development of endemic S strain transmission between cattle.

6 Success in achieving objectives

Summary of activity undertaken
Collection of the blood, faecal and tissue samples as proposed has been completed and all the cattle have been removed from the property (as of the 21st November, 2006). Analysis of testing data and herd/flock management information has been provided.

Specific Objectives

- Describe in detail the history of infection and management of the resident sheep flock

- Describe in detail the history of infection and management of the beef herd

Detailed information on calving and grazing records was difficult to obtain as herd manager did not keep records of sufficient quality of quantity. Good summary information on the disease history and management procedures, and a detailed property map have been provided. Together these provide information that partially fulfils this objective.

- Describe the within herd distribution and prevalence of infection with S strain *Mptb* in different cohorts (age/sex/origin/lineage) within the herd.

This objective has been fully achieved. In addition, this study has provided the first documented estimates of the sensitivity of diagnostic tests for S strain *Mptb* infection in cattle.

- Provide a better understanding of the epidemiology of S strain *Mptb* infection in cattle and assist in the development of control strategies for other similarly affected herds.

This objective has been achieved as far as is possible given that we have investigated only one, heavily infected herd.

- Attempt to identify likely risk factors for infection of the herd and cohorts within the herd.

This objective has been achieved as far as is possible given the lack of detail available regarding herd/flock management and that we have investigated only one, heavily infected herd.

- Attempt to determine if S-strain *M paratuberculosis* has been transmitted between cattle within the herd.

The high prevalence of infection and evidence for on-going transmission in, or to, the cattle herd despite apparent control in the resident sheep flock suggests that cattle to cattle transmission should be considered possible if not probable. This objective has been achieved as far as is possible.

7 Impact on meat and livestock industry – now and in five years time

This study has provided detailed insight into the prevalence and distribution of S strain *Mptb* infection in a single, heavily infected beef herd in Victoria. It also provides the first documented estimates of the sensitivity of diagnostic tests for S strain *Mptb* infection in cattle.

The results of this study challenge a number of current assumptions about S strain *Mptb* infection in cattle.

- It is generally assumed that cattle are not readily infected with S strain *Mptb*. The finding that at least 19.2% of the herd are infected suggests that given favourable circumstances cattle may be readily infected but may not be **detected** as infected unless post-mortem testing is undertaken.
- It is generally assumed that infection does not progress to clinical disease in cattle. The herd under examination in this study experienced one confirmed clinical case and two other probable clinical cases in under 12 months.
- Finally, it is assumed that cattle to cattle transmission of S strain *Mptb* does not occur. The high prevalence of infection and evidence for on-going transmission within, or to, the cattle herd despite apparent control in the resident sheep flock suggests that cattle to cattle transmission should be considered possible if not probable.

The most significant impact from this work will be the implications for the current Johne's disease control and accreditation programs in south eastern Australia. Historically, it has been assumed that cattle do not become clinically affected by Johne's disease when grazing with *Mptb* infected sheep for extended periods and that cattle to cattle transmission of S strain *Mptb* does not occur. Many properties undertaking OJD control programs run cattle as an additional or alternative enterprise. Although S strain *Mptb* infection is a sporadic and relatively rare event, changes to the use of alternative species for pasture decontamination are warranted.

This study supports new recommendations that

- When cattle are being reared in OJD endemic areas care should be taken to ensure that they are not exposed directly or indirectly to infected sheep or manure (including contaminated run-off from neighbouring properties) until the cattle are at least 12 months old.
- Care should be taken when hand feeding cattle to avoid areas of high sheep manure build up.
- Diagnostic testing of suspect clinical cases should routinely involve strain typing of any cultivated *Mptb*.
- Disease control and accreditation programs for both OJD and for bovine Johne's disease should include consideration of the possibility that S strain Mptb may be transmitted to cattle

Finally, even if only small percentage of the 20,000 herds in Victoria are exposed to sufficient Mptb challenge to allow disease transmission to the cattle it is likely that the prevalence of Johne's disease in beef herds will increase and that the relative frequency of infection with S strain *Mptb* will increase. **Active management to protect the beef industry now may prevent many new cases of Johne's disease and reduce the potential for development of endemic S strain transmission between cattle.**

Conclusions and recommendations

- Diagnostic testing of suspect clinical cases of bovine Johne's disease should routinely involve strain typing of any cultivated *Mptb*.

- Existing diagnostic tests may be used to detect cattle infected with S strain Mptb. Strain typing is required to distinguish C and S strain infections.

- Ante-mortem tests (ELISA and faecal culture) have poor sensitivity except when applied to cattle in the latter stages of disease pathogenesis (5 years old and older).

- The true prevalence and distribution of S strain Mptb infection in a herd is therefore difficult to estimate using existing ante-mortem diagnostic tests.

- The on-going source of infection of cattle in this study remains unclear but could include infected and shedding adult cattle, infected and shedding sheep resident on the study property or organisms in the environment originating from contaminated water draining from neighbouring OJD affected properties.

- Disease control and accreditation programs for both OJD and for bovine Johne's disease should include consideration of the possibility that S strain *Mptb* may be transmitted to cattle

- Taking the most cautious approach, only adult cattle should be grazed on areas recently grazed by known OJD infected sheep or contaminated by drainage from adjacent landholdings that graze sheep known to be infected with *Mptb*.

- Active management to protect the beef industry now may prevent many new cases of Johne's disease and reduce the potential for development of endemic S strain transmission between cattle.

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