final report

Project code: **P.PSH.0301**

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Date published: **October 2011**

ISBN: **9781741916485**

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Ovine strain of *Mycobacterium paratuberculosis* in beef cattle: A Case Study #2

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

In 2007 a Monitored Negative 2 (MN2) Cattle Market Assurance Program accredited stud beef herd in the Ballarat region was identified as infected with ovine (S) strain *Mycobacterium avium* subsp. *paratuberculosis (Mptb)*. There was no history of exposure to an infected sheep flock, either on or adjacent to the affected property.

To determine the within herd distribution and prevalence of infection, and in an attempt to identify risk factors for infection and transmission, samples from the 55 head herd have been examined for the presence of *Mptb*. No further infection with *Mptb* was detected, nor within herd spread. Further testing of cattle enterprises in ovine Johne's disease prevalent areas would be required to evaluate the significance of S strain infection in cattle, although it is likely to be rare.

Executive Summary

Johne's disease (JD) is a chronic gastrointestinal wasting disease of some importance in cattle and sheep populations, caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Mptb*), which can survive in the environment for months[1].

There are distinct strains of *Mptb*; a cattle (C) strain that affects predominantly cattle (but is also capable of infecting goats, deer and alpaca), and a sheep (S) strain predominantly affecting sheep[2]. Retrospectively, *S* strain *Mptb* has been diagnosed in cattle on at least eight New South Wales (NSW) properties, and in 1999 and 2005 cattle with clinical signs were diagnosed with *S* strain *Mptb* in Victoria. These properties had known ovine Johne's disease (OJD) infection in sheep.

This study was undertaken following the diagnosis of *S* strain *Mptb* infection a seven year old cow in a MN2 stud beef herd in the Ballarat region of Victoria that had been managed in accordance with the National Market Assurance Program for Cattle (CattleMAP)[4] on a property without sheep.

The Australian Johne's Disease Market Assurance Program, an audited quality assurance program incorporates animal health risk assessment, testing and movement control that provides low risk animals for the various industry sectors.

To determine the within herd distribution of *S* strain *Mptb*, to improve understanding of S strain *Mptb* infection, and to examine possible risk factors for disease transmission, the remaining 55 head of cattle were slaughtered and samples collected and examined for *S* strain *Mptb* .

The index case was the only animal found to be infected in the herd, and the route of infection could not be determined given the results. There was no evidence of within herd spread of infection, which is more significant as time increases since initial infection of the index case.

Contents

1 Abbreviations

BJD	Bovine Johne's disease
CattleMAP	Johne's Disease Market Assurance Program for Cattle
DPI	Department of Primary Industries
ELISA	Enzyme Linked Immunosorbent Assay
GVP	Gribbles Veterinary Pathology
JD	Johne's Disease
MLA	Meat and Livestock Australia
MN#	CattleMAP herd status
Mptb	*Mycobacterium avium* subsp. *paratuberculosis*
OJD	Ovine Johne's disease
PCR	Polymerase Chain Reaction

2 Background

Johne's disease is a chronic granulomatous enteritis of ruminants and camelids caused by Mycobacterium avium subspecies paratuberculosis (*Mptb*). In cattle, the incubation period is long, with disease manifesting as chronic diarrhoea and severe wasting in mature animals. Young cattle, less than 12 months of age, are most susceptible to infection, usually via the faecal-oral route, with very large numbers of organisms shed into the environment in the faeces of clinically affected animals. Subclinical animals also can shed organisms.[5]

It is recognised that there are two distinct strains of *Mptb* affecting cattle, goats and deer (the C strain) and a S strain affecting sheep[6,7]. The strains can be differentiated by culture or with Polymerase Chain Reaction (PCR) technology. Experience indicates that cattle co-grazing with OJD infected sheep for extended periods usually do not become clinically affected with Johne's disease; an important basis of existing management strategies. However cases in Australia in the past decade have demonstrated that in some circumstances this species-strain adaptation or preference is not absolute.

There have been a few instances where *S* strain *Mptb* has been diagnosed in cattle, including through clinical investigation, retrospective diagnosis from archived histological sections and during testing undertaken as part of Australian Johne's Disease Market Assurance Program for Cattle (CattleMAP). Exposure to infected sheep flocks during the 1990s has been identified in an area where OJD is endemic and of relatively high prevalence (10% of flocks known to be infected)[3,8].

Recently, cattle in two beef herds in the Ballarat region have been found to be infected with *S* strain *Mptb.* One property, the subject of study MLA 206[2], had a history of severe OJD in its flock over 1998 to 2002. A prevalence of greater than 50% was detected in an abattoir consignment, and flock mortality reached 15-18%.

The beef herd in this case report had no history of OJD and no clear external source of infection. The seven year old index cow was diagnosed following a positive Enzyme Linked Immunosorbent Assay (ELISA) result on the herd's third CattleMAP herd serology test. Prior to this, the herd had achieved a Monitored Negative 2 (MN2) status in the CattleMAP program. CattleMAP is designed to reduce the risk of bovine Johne's disease (BJD) on properties through risk based management, including property assessment, implementation of a biosecurity plan and controlled introductions of stock, and testing involving series of negative herd ELISA tests.

This project was undertaken to determine the within herd distribution of *S* strain Mptb infection in a CattleMAP herd from Ballarat, to provide a better understanding of the epidemiology of *S* strain Mptb infection in cattle.

3 Project Objectives

To report the findings of a detailed investigation in a beef herd infected with *S* strain *M. paratuberculosis*, specifically:

1. Describe in detail the history of infection and management of the beef herd.

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

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

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

5. Assess environmental contamination with *S* strain *M. paratuberculosis* and attempt to establish the source of that contamination.

4 Methodology

4.1.1 Study Design

This is a cross-sectional study of the herd; the owner of the affected herd elected to remove to 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 of slaughter. Samples were processed at a veterinary laboratory and examined for antibody, histological or cultural evidence of *Mptb* infection, in particular for presence of the *S* strain of *Mptb*.

Herd profile information, rainfall records and district records for nearby properties known to be infected with OJD were examined.

4.1.2 Sample collection and processing for serological testing

Blood samples were collected from the tail vein using plain Vacutainer®[1] tubes, allowed to clot at room temperature and then packaged for transport to Gribbles Veterinary Pathology (GVP). On arrival the serum was tested using the BJD absorbed ELISA for antibodies against *Mptb*[9].

4.1.3 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 sample to prevent cross-contamination. Samples were chilled and sent to GVP within 12-24 hours of collection.

At GVP, the samples were subjected to the following five processing steps[9]

1. Decontamination of the specimen in hexadecylpyridinium chloride (HPC).
2. Liquid media culture using a BACTEC®[2] 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. Polymerase Chain Reaction (PCR) on liquid media from step 2, 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.

4.1.4 Slaughter and post-mortem examination

All cattle in the herd were slaughtered at abattoirs in four consignments between 3 May and 22 May 2007. Each was examined by the project manager or assisting Department of Primary Industries (DPI) Animal Health Officers. A standard set of seven sites were sampled as outlined in the Australian Cattle MAP guidelines[4].

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

[1] Becton Dickinson Company, Australia
[2] Becton Dickinson Company, Australia

4.1.5 Sample collection and processing for histopathology

Once collected, one set of tissues were fixed in 10% buffered formalin and sent to GVP for processing and examination. Samples were embedded in paraffin, stained for normal tissue elements with haematoxylin and eosin and also for acid fast bacilli using the Ziehl-Neelsen method[9].

4.1.6 Sample collection and processing for bacteriological culture of tissue

The other set of tissues were submitted fresh to GVP for processing and examination. Bacteriological culture was performed using the same steps as described in 4.1.3 for faecal culture.

4.1.7 Sample collection and processing for bacteriological culture of soil

Fifty grams of soil were collected from 10 sites on the property, as indicated on the map (see figure 3). Two samples were taken from paddock six where the index case was kept prior to slaughter. Samples were submitted to the DPI laboratory at Attwood.

Two grams of soil were suspended in 10 millilitres of sterile saline (0.9% sodium chloride). After mixing, the suspension was allowed to settle for at least 30 minutes. Five millilitres of the supernatant was transferred into a 25ml centrifuge tube containing 20ml of 0.95% hexadecylpyridinium chloride in half-strength brain heart infusion (HPC/BHI). The mixture was incubated for 18 to 24 hours at 37^{o}C then centrifuged at 900G for 30 minutes. The supernatant was discarded and pellet resuspended in 1 millilitre of an antibiotic cocktail containing 100 µg of each of vancomycin, amphotericin and nalidixic acid in half-strength brain heart infusion (VAN/BHI). After the incubation for 72 hours at 37^{o}C, 0.2 millilitres of the suspension was inoculated into a BACTEC 12B bottle supplemented with 1mL egg yolk, 5µg mycobactin J, 0.2 millilitres PANTA and 0.7 millilitres of water. The BACTEC primary cultures were incubated at 37^{o}C and monitored for growth for at least 12 weeks. Cultures showing growth were subcultured to Herrold's egg yolk medium (HEY), Herrold's egg yolk medium with mycobactin (HEYM) and BACTEC 12B bottle supplemented with 5µg mycobactin J and 0.2 millilitres PANTA and tested using the IS900 PCR[12]. The identification of M. paratuberculosis was based on demonstration of mycobactin dependency and results of the IS900 PCR[10].

4.1.8 Herd management data collection

Face to face interviews were conducted with the herd owners. Records of lineage and ages of all cattle slaughtered were provided. Daily rainfall records for the property in 1998 an 1999 were provided. DPI Animal Health District records were searched for locations of properties known to be infected with OJD. Telephone conversations with the private veterinarian servicing the area were made regarding health of neighbouring sheep flocks.

5 Results

4.1.1 Testing Outcomes

In total, 56 head of cattle including the index case were sampled before and after slaughter.

The index cow returned positive results to ELISA blood testing, histopathology and *S* strain *Mptb* was identified by faecal and tissue culture, PCR and solid media culture, (see Figure 1).

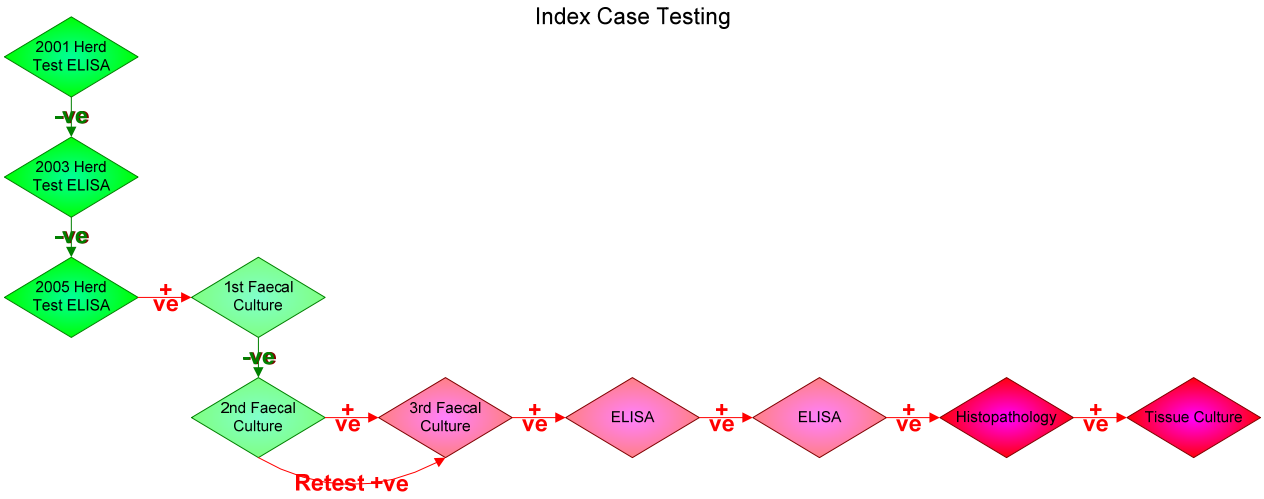

Figure 1. Index cow test results summary

In October 2005 a faecal sample was collected and submitted for culture with a negative result. In accordance with CattleMAP requirements (see Figure 2), in April 2006 a second faecal sample was submitted but returned a positive culture for the *S* strain of *Mptb*, verified by PCR and solid media culture. A third faecal sample and two blood samples were submitted in July 2006. Both blood samples returned positive ELISA results and the faecal culture again returned a positive result for *S* strain *Mptb*, verified by PCR and solid media culture.
Consequently in March 2007 the index cow was slaughtered and samples were submitted for histopathology and culture, as per routine. Histologically there was granulomatous inflammation and acid fast bacteria identified with the Ziehl-Neelsen stain and the tissue culture was positive for *S* strain *Mptb*.

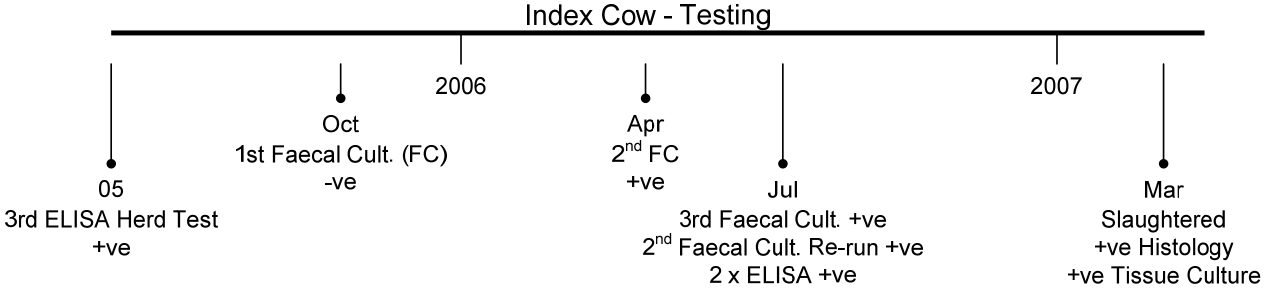

Figure 2. Index cow testing history after positive herd test

The remaining 55 head aged between 3 months and 11 years returned negative results to ELISA tests, faecal and tissue cultures and histopathology examination.

Ten soil samples cultured for *Mptb* returned negative results. See table 1.

Table 1. Environmental testing results

Sample site	*Result*
01. Yards	*Negative*
02. Paddock 6 Gateway	*Growth of micro-organisms other than Mptb detected.*
03. Paddock 6 Near trough	*Growth of micro-organisms other than Mptb detected.*
04. Paddock 7	*Growth of micro-organisms other than Mptb detected.*
05. Paddock 10	*Negative*
06. Paddock 11 Dam bank	*Negative*
07. Paddock 12	*Growth of micro-organisms other than Mptb detected.*
08. Paddock 1	*Negative*
09. Paddock 2	*Negative*
10. Paddock 3	*Negative*

4.1.2 Property location and description

The study property comprised 56 hectares located 38 km from Ballarat in a south easterly direction. Figure 3 is an aerial map of the property showing the major topographical features of the property, direction of drainage onto the property and the enterprise type of neighbouring properties.

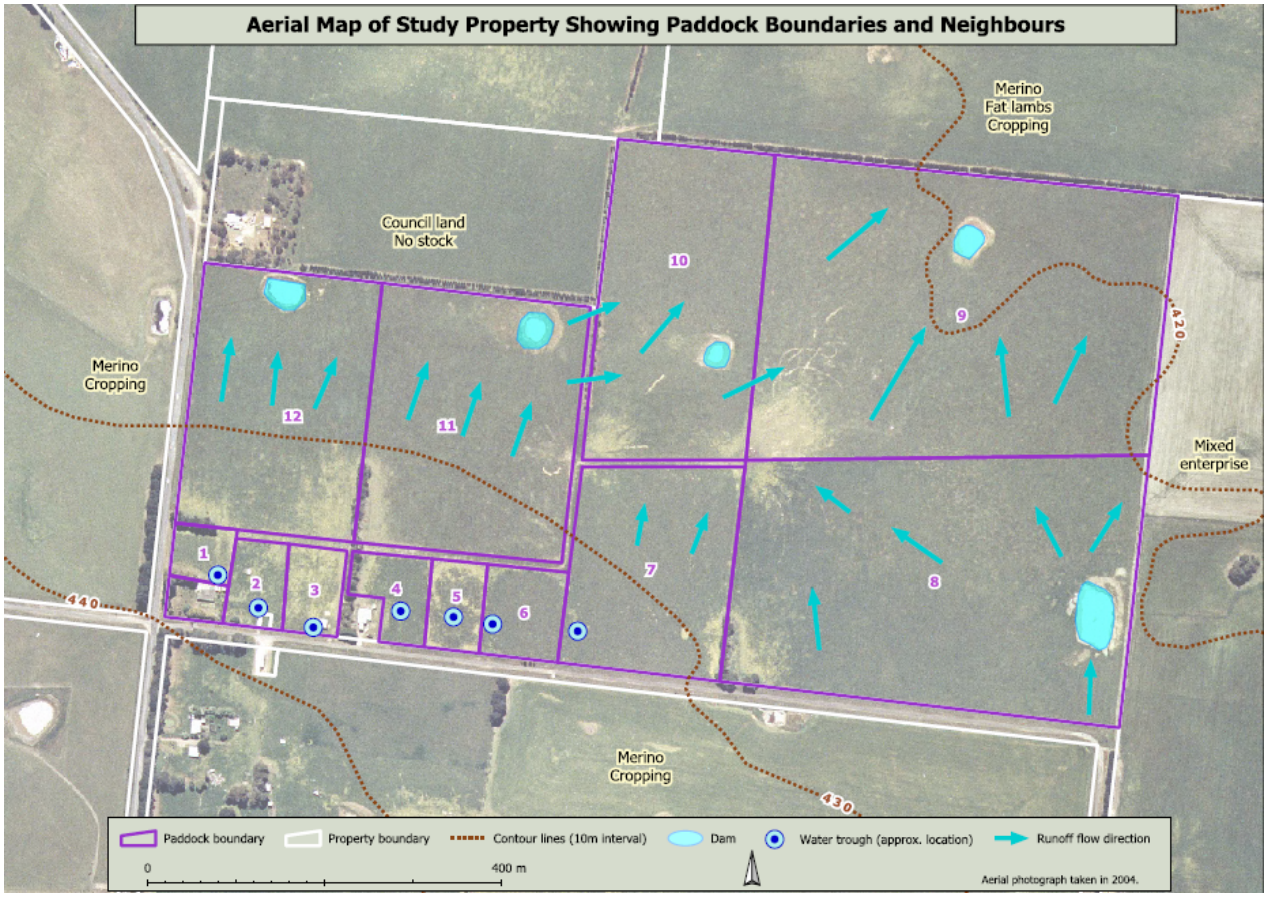

Figure 3. Aerial map of study property showing paddock boundaries, drainage, water points and neighbours

The property is subdivided into 12 smaller paddocks of improved pasture on grey loam soil.

Water supply for the paddocks comprises town water for the six smaller paddocks close to the house, and dams for the remaining paddocks. The property is on a shallow north facing slope with a tarmac road on the southern and western borders. The possibility of run-off reaching paddock eight was documented by the private practitioner managing the CattleMAP program for the property and no cattle under the age of one year were permitted to graze there from 2001.

The property has been held by the current owners for 22 to 23 years. No sheep have been on the property since a year prior to purchase in 1985.

Neighbouring properties to the north, south and west run sheep and cropping enterprises, and are not known to be infected with Johne's disease.

4.1.3 Examination of herd records

At the time of slaughter, two-thirds (37) of the herd comprised of animals younger than four years old (see Figure 4).

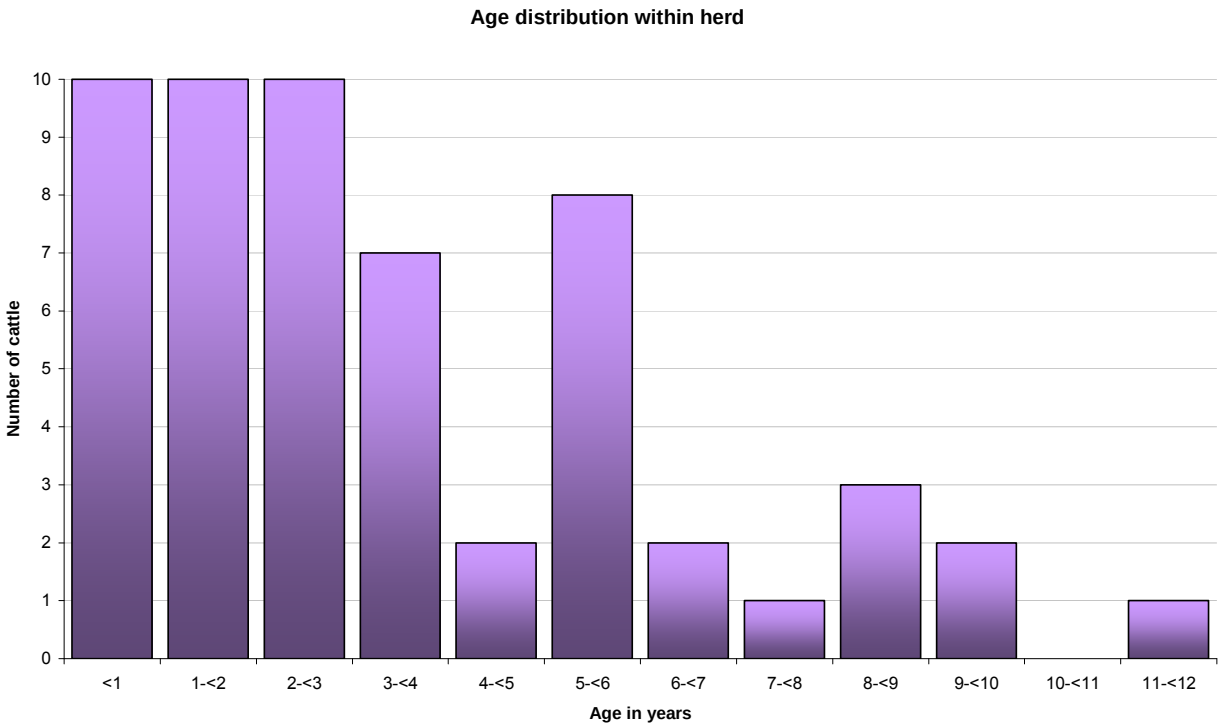

Figure 4. Age structure of the herd

Records detailing family trees and dates of birth were examined, some of which are detailed in Figure 5, along with the herd testing history. In 2001 the herd was sample tested in accordance with the requirements for CattleMAP, returning negative results allowing the herd to achieve a Monitored Negative 1 (MN1) status. MN2 status was given based on herd testing undertaken in 2003. The index case was detected at the 2005 testing, which would have seen the property attain a MN3 status if testing had been negative.

Two daughters born in 2001 and 2005 to the index case and three of their offspring were slaughtered and returned negative results.

The dam of the index case was bred on the property but slaughtered prior to the study without testing and without signs of disease.

A cohort of the index case and her three offspring were slaughtered and returned negative results, as did the only animal older than the index case (an 11 year old cow).

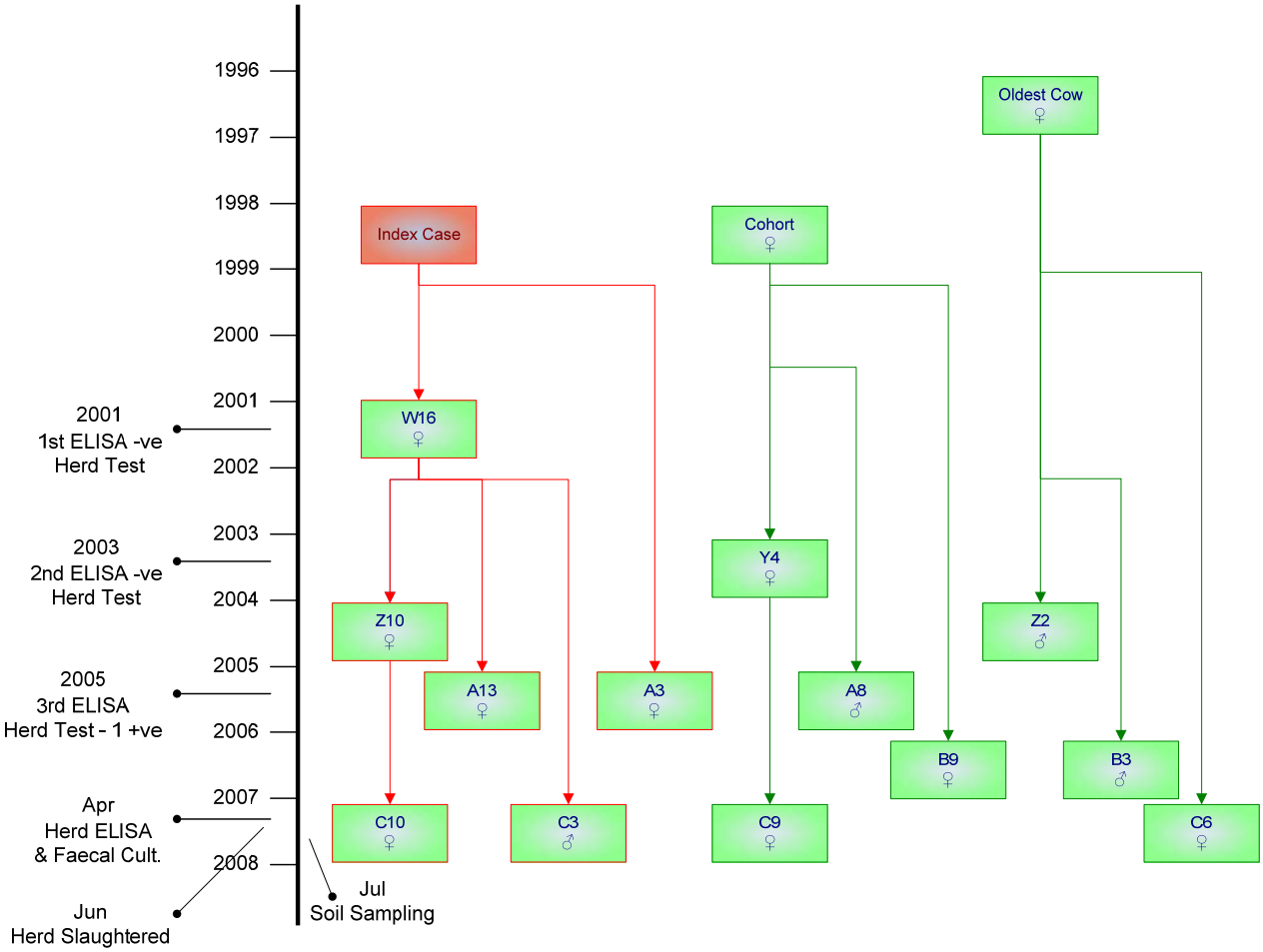

Figure 5. Family lineages and herd testing histories for the herd

This has been a closed stud herd since 1998. The last introduction was a BJD ELISA negative bull in December 1998, which left in May 1999. There is no known history of suspicion of infection on any property to which stud cattle have been sold.

4.1.4 Assessment of locations of properties known to be infected with OJD.

There are no properties known to be infected with OJD bordering the index farm currently or historically. Neither have there been any abattoir traces to those properties, which comprise cropping and merinos. Compulsory Johne's disease testing of neighbours is not an approach used in Victoria, and the majority of properties have not been assessed. There are a number of infected properties in Moorabool Shire, where the property is located, and the neighbouring Golden Plains Shire.

Telephone conversation with private veterinarian regarding health of neighbouring sheep flocks revealed no suspicion or evidence for infection with OJD.

4.1.5 Analysis of rainfall

In 1997 the year prior to the birth of the index cow was the driest for 15 years, with rainfall below the first decile. The rainfall records for 1998 and 1999, during the time the index case was younger than 12 months, were examined, These years had rainfalls below the fifth decile. November 1998 was the wettest month for the period (see Figure 6), and the only month to experience daily rainfall in excess of 17 millilitres, which occurred on three days (see Figure 7).

One day in November experienced a relative deluge with 104.5 millilitres recorded. This occurred when the index case was seven months old and could have been sufficient to wash potentially infectious sheep faecal pellets onto the property. In the 1999 period, only two days had rainfall over 20 millilitres.

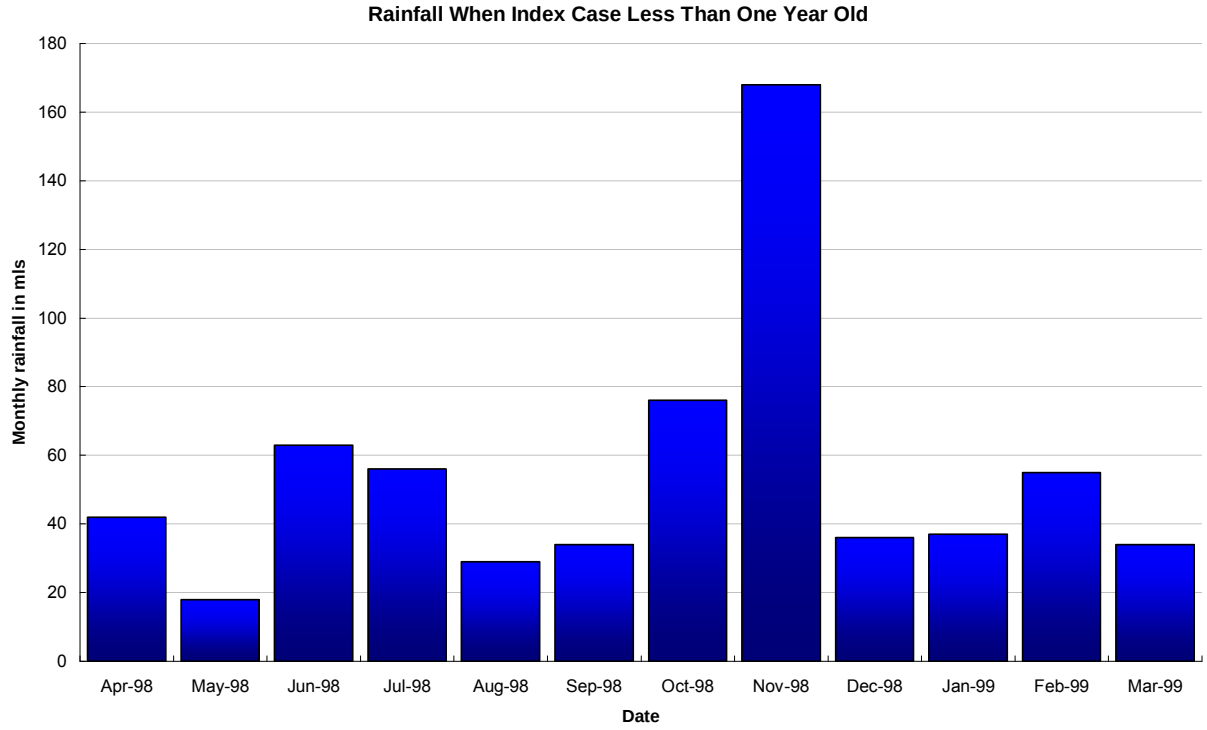

Figure 6. Monthly rainfall totals for the period April 1998 to March 1999

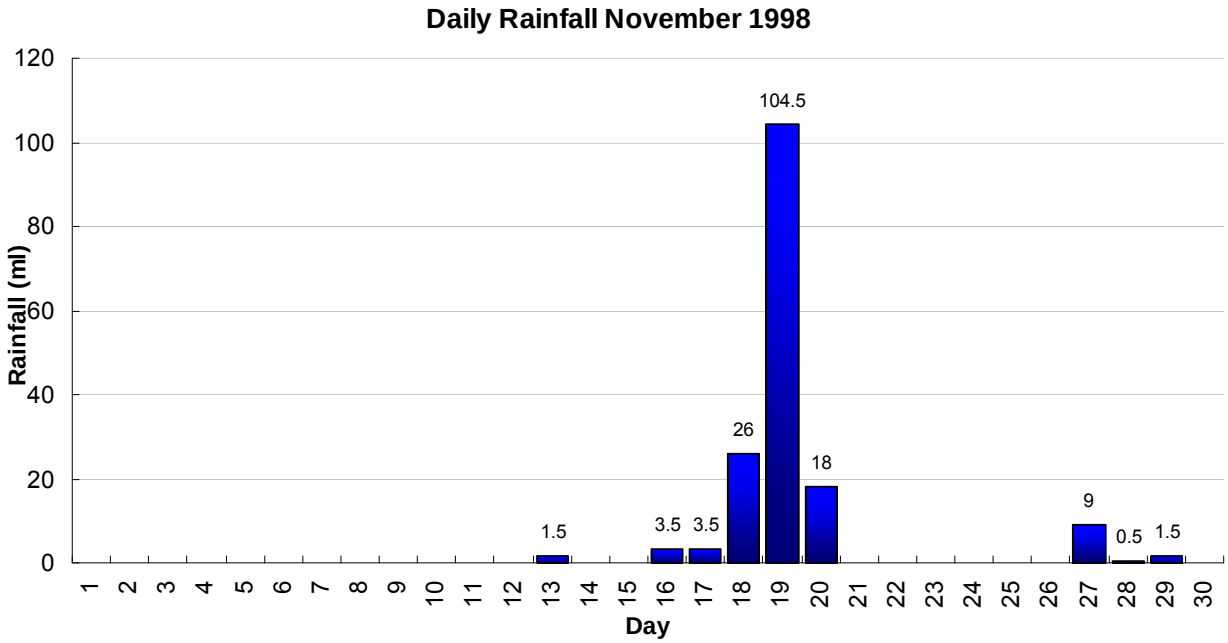

Figure 7. Daily rainfall totals in November 1998

6 Discussion

This study failed to determine the source of infection with *S* strain *Mptb* of the one (index) infected cow, despite extraordinary levels of testing undertaken possible with slaughter of the herd. There is no evidence that infection had spread within the herd, vertically or horizontally, although the status of the dam of the index cow could not be determined. The index cow was shedding *Mptb*, as evidenced by positive faecal culture, but this was not detected in soil samples taken from the pasture. Cattle are not readily infected with *S* strain *Mptb;* there is an extremely low prevalence in beef cattle evidenced by strain typing of culture positive herds. Where it occurs, it rarely progresses to clinical disease in cattle, and cattle to cattle transmission of *S* strain *Mptb* has not been confirmed. This study does not contradict this situation. Finding no evidence of *Mptb* in the environment suggests low levels of infectious load for the remainder of the herd

There is the possibility that infection may have spread if the index cow had been allowed to remain on the property for longer so increasing the infection pressure on the remainder of the herd, although this is unknown. This is somewhat in contrast to the findings of Fahy and Ridge[2] where three clinical cases occurred and a further 14 out of 73 cattle aged 15 months to 6 years were found to be infected.

The significance of the negative soil tests is debatable, although combined with lack of disease spread within the herd and low level of faecal shedding indicated low levels of environmental contamination.

There is a possibility that the high rainfall experienced in the November 1998, when the index case was approximately seven months old, could have washed infected sheep faecal pellets from a neighbouring property onto this property. The surrounding sheep properties are not, and have not been, known to be infected with OJD, nor are subject to suspicion of disease by their local veterinarian. However, compulsory Johne's disease testing of neighbours is not an approach used in Victoria, consequently the involvement of neighbours cannot be further elucidated.

The index cow in this case study did not exhibit clinical signs and probably would not have been diagnosed if the producer had not elected to enter the CattleMAP program

This study further highlights the deficiencies of existing tests for Johne's disease. Nationally, histopathology and tissue culture are considered the gold standard test. The ELISA test has well known limitations, especially its poor sensitivity which does improve with age. The index case was not identified until her third test at seven years of age. This matches with the experiences of the Victorian Test and Control Program, for which the average reactor age was 5.7 years in 2004-05[11]. ELISA specificity is very high, which was not contradicted in this study. Not unexpectedly, faecal culture correctly identified infection in the two latter of three cultures of the index case. Sensitivity also increases with stage of infection as intermittent shedding becomes more frequent and persistent.

7 Success in Achieving Objectives

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

  This objective has been achieved. The history of the process leading to the diagnosis of the index case and the management of the stud herd has been described in detail.

- 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 achieved. No additional cases other than the index case were identified. Meticulous lineage details have been kept by the producer enabling family tree of index case and cohort to be plotted.

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

  This objective has been achieved although no risk factors could be clearly identified, especially given that the 55 head slaughtered all returned negative results.

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

  This objective has been achieved. No S-strain Mptb has been transmitted between cattle within the herd.

- Assess environmental contamination with S strain Mptb and attempt to establish the source of that contamination.

  This objective has been achieved. Soil sample from the property were cultured and found to be negative for Mptb.

Impact on Meat and Livestock Industry – now & in five years time

- This study has confirmed findings of other reports, that cattle may sporadically become infected with S strain *Mptb*.

- This study did not find evidence of spread of the S strain *Mptb* within the herd, and that infection was at a very low level, suggesting that the herd was not exposed to significant contamination with *S* strain *Mptb* or that cattle are inherently resistant.

8 Conclusions and Recommendations

- The prevalence of transmission of *S* strain *Mptb* to cattle is unknown but likely to be low.
- Cattle to cattle transmission has not occurred in this case.
- Industry actively pursues the development and adoption of further tools that producers can practically use to manage BJD in cattle, including assessing the role of vaccination.
- Consideration be given to encouraging producers co-grazing sheep and beef cattle to vaccinate their sheep as a potential means of protecting their cattle from S strain infection.

Acknowledgements

The authors would like to thank the affected producers whose co-operation with access to stock and provision of records has allowed this project to proceed; to Linda Fahy and Sally Ridge for sharing of knowledge and reference material from MLA 206 study and all Department of Primary Industries district staff for their willing participation collecting samples.

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