MEAT & LIVESTOCK
AUSTRALIA

On farm

Cross Species Transmission of Ovine Johne's Disease – Phase 2 Cattle

Project number OJD.016
Final Report prepared for MLA by:

B.J. Moloney and R.J. Whittington
NSW Agriculture
Locked Bag 21 Orange NSW 2800
02 6391 3687

Meat and Livestock Australia Ltd
Locked Bag 991
North Sydney NSW 2059

ISBN 1 74036 109 1

September 2002

Animal Health and Welfare

ABSTRACT

One thousand seven hundred and seventy four cattle from 12 properties were sampled by ELISA and faecal culture to detect Johne's Disease. All properties had a known significant history of Johne's disease in sheep. They were mostly selected from participants in NOJDP Trial 1.1 (a study to examine the effectiveness of an eradication strategy) and had cattle known to be susceptible to JD at the time that their properties were infected with OJD. All gave negative results on serology; only one animal from a herd of 349 gave a single positive faecal culture result, with all follow-up investigations being negative, suggesting passive transfer of the organism. Due to the small size of some of the herds tested, and the fact that no confirmed infected animals or herds were detected, it is not possible to give maximum estimate of the prevalence of OJD in exposed susceptible cattle. However, using information derived from previous investigations and some additional results in the addendum to this project report, it is known that there are at least 6 cattle herds infected with "S"strain, in NSW, at the present time.

EXECUTIVE SUMMARY

Australian JD control and assurance programs assume that sheep and cattle strains of *M paratuberculosis* are epidemiologically distinct infections.

This project was undertaken to determine the extent to which *Mycobacterium avium* subsp. *paratuberculosis* S strain (OJD) can infect and/or be passively transferred by cattle.

In this study, 1774 cattle on 12 OJD-infected properties (range 90-985 head on each farm) were sampled by serology and faecal culture. All cattle were home-bred on the properties and had thus been exposed to OJD as susceptible calves. Testing was undertaken on the cattle at age 2 years or more. All animals were negative for serology. One property had a single animal with one faecal culture positive (S strain) result, but follow-up serology, faecal culture, post-mortem examination, histopathology and tissue culture were all negative, suggesting passive transfer of the organism (less than 12 months since last infected sheep was on the property) or very early infection.

Management routines that were followed on these properties enabled direct (cattle co-grazing with sheep), and indirect contact to occur (cattle following sheep and sheep following cattle).

An addendum to this report describes additional occurrences of OJD transmission to cattle. During the course of the study, 2 additional properties undergoing Cattle MAP testing each had 2 serological reactor animals which all gave follow-up faecal culture positive results (S strain). Three of these animals were subsequently diagnosed as histologically positive for Johne's disease. Finalisation of investigation is still pending for the fourth animal. One of these properties did not have sheep present on it, but had infected neighbours adjacent to the calving paddock. The other property was outside the known OJD infected areas, but had raised heifers from weaning to springing on a known OJD infected property. These observations confirm existing beliefs about the risk of transmission of OJD to cattle, i.e. that the risk of transmission is low except where young cattle are reared in contact with infected sheep.

The prevalence of disease in cattle exposed to sheep infected with OJD was assessed as low, but could not be precisely determined. One estimate would be up to 0.4% to 0.6% of cattle running on OJD infected properties may be infected with *M paratuberculosis* (S strain), based on 1 culture positive out of 1744 animals tested. What is known at present is there are at least 6 cattle herds in NSW infected with OJD.

The likely risk factors associated with cattle developing infection or passive transfer of OJD could not be determined from this study due to the absence of disease. However, in the properties reported in the addendum, exposure to calves may occur due to run-off from infected neighbouring property(s) or from hand feeding sheep and cattle together during drought.

TABLE OF CONTENTS

1. MAIN REPORT 4

1.1. Literature review 4

1.1.1.Review of literature since November 1999, adding to evidence for cross-species transmissions between sheep and cattle 7

1.1.2.Evidence against cross-species transmission between sheep and cattle 8

2. BACKGROUND AND INDUSTRY CONTEXT 8

3. PROJECT OBJECTIVES 9

4. MATERIALS AND METHODS 9

4.1. Study design 9

4.2. Laboratory and other methods 9

4.2.1 Serology 9

4.2.2 Faecal culture 9

4.2.3 Post -mortem examination 10

4.2.4 Tissue culture 10

4.2.5 Identification and typing 10

4.2.6 Histopathology 10

5. RESULTS 11

6. DISCUSSION 15

6.1. Estimated within herd prevalence of OJD in cattle exposed to OJD 16

6.2. Estimated herd (area) prevalence of OJD in exposed cattle 17

6.3. Risk factors associated with cattle developing infection or passive transfer of OJD 17

6.4. The need for longer term study 17

6.5. Additional Validation of the ELISA for suitability as a screening test for CattleMAP 18

7. SUCCESS IN ACHIEVING OBJECTIVES 18

8. IMPACT ON MEAT AND LIVESTOCK INDUSTRY 19

9. CONCLUSIONS AND RECOMMENDATIONS 19

ACKNOWLEDGEMENTS 19

BIBLIOGRAPHY 20

1. MAIN REPORT

1.1. Literature review

Following extensive surveys in south eastern Australia it seems clear that ovine and bovine forms of Johne's disease are mostly caused by distinct strains of *M. avium* subsp. *paratuberculosis* (Cousins *et al* 2000; Whittington *et al* 2000). However, the detection of sheep strains of *M. paratuberculosis* in cattle on several farms with ovine Johne's disease in Australia (Whittington *et al* 2001) has raised the possibility that successful control of this disease could be compromised by reservoirs of infection in other species. The success of depopulation and decontamination on farms with multiple grazing species depends on the segregation of infection within individual species and may also depend on the absence of wildlife reservoirs. In Australia, where sheep and cattle are the predominant grazing species and where paratuberculosis is believed not to be spread commonly between these species, a management option has been to use cattle to graze pastures during the decontamination period after depopulation of paratuberculous sheep.

Besides their role in compromising decontamination of OJD for sheep, as happened in Iceland (Fridriksdottir *et al*, 2000), cattle may have the potential to spread infection in an on-farm control program that is relying on grazing management or vaccination. Alternatively (although outside the scope of this project) OJD infected sheep may compromise the control that is being sought of paratuberculosis in cattle.

Ian Morgan's review in 1999 (Appendix 5) assessed the available evidence about cross-species infections with *M. paratuberculosis* and the likely effect on the success of paratuberculosis control programs. He considered four types of evidence of cross-species infection with *M. paratuberculosis:*

- Reports of natural infections with *M. paratuberculosis* in species other than cattle, sheep and goats
- Experimental infection studies with *M. paratuberculosis*
- Epidemiological evidence of possible transmission of *M. paratuberculosis* between species
- Molecular epidemiology studies

but did not reach unequivocal conclusions.

Whittington *et al* (2000) reviewed molecular and epidemiological evidence, which is reproduced below from that paper. For a listing of references see Appendix 6.

It has been known for many decades that there are at least two types of *M. avium* subsp. *paratuberculosis.* A less readily cultivated type is the common but not invariable cause of paratuberculosis in sheep, while another readily cultivated type is the most common cause of the disease in cattle. The latter type is frequently associated with infection in other species that graze in contact with cattle but may also occur in sheep (23, 31, 32, 33). Evidence of the difficulty of culture of *M. avium* subsp. *paratuberculosis* from sheep with paratuberculosis comes from a variety of countries and is so consistent that there can be no doubt about phenotypic strain differences [reviewed in (45)].

There is somewhat less certainty regarding the true degree of host adaptation or preference of these phenotypically distinct strains. In Scotland, ovine paratuberculosis was most often attributed to pigmented, poorly-growing mycobacteria. Non-pigmented and readily-cultivated strains similar to those commonly obtained from cattle were also isolated from sheep in Scotland but only from a minority of farms (37, 38). The pigmented ovine strains appeared to be more pathogenic for sheep than the classical non-pigmented bovine strains (35). In Iceland, ovine paratuberculosis was caused by essentially non-cultivable mycobacteria and prior to a compulsory vaccination program was associated with significant mortality rates, though the infection in cattle due to these strains tended to be subclinical (15, 19). There are reports from England, Australia and New Zealand that paratuberculosis was not diagnosed in cattle in contact with paratuberculous sheep (2, 23, 34) and a report of apparent failure of natural transmission of

the disease to sheep that were exposed to paratuberculous cattle (2). Conversely, a sheep on a cattle farm was found to be infected with a cattle strain (28), rotational grazing of sheep with paratuberculous cattle resulted in infection in 4 of 6 sheep in a study in New Zealand (32) and infection was probably transmitted to a sheep by close contact with paratuberculous cattle in Australia (22).

These observations appear to have led to a common belief in Australia that sheep may succumb to infection with either strain and that cattle are not particularly susceptible to the strains that cause the predominant form of ovine paratuberculosis. However, there is one report of natural paratuberculosis in a cow in Scotland due to a pigmented ovine strain (41) which was then transmitted experimentally back to sheep, and several reports in which young cattle succumbed to paratuberculosis following experimental dosing with either pigmented or 'Icelandic' organisms derived from sheep (23, 39). In Iceland, epidemiological observations are consistent with *M. avium* subsp. *paratuberculosis* having been introduced to the local sheep population with imported European sheep, its spread to the local cattle population in which it became endemic, and its subsequent transmission from cattle back to sheep after an ovine depopulation and restocking program (15, 27).

In summary, solid evidence of host specificity for the phenotypically defined sheep and cattle strains of *M. avium* subsp. *paratuberculosis* is lacking. Massive doses of the organism sufficient to overwhelm normal host defences have been used in experimental infections so that the conclusions may not be applicable in the field, while epidemiological evidence for the segregation of strains within ruminant hosts can be contrasted with epidemiological evidence of transmission between these hosts.

In addition to phenotypic data and epidemiological observations, there is clear evidence at the molecular level of heterogeneity among isolates of *M. avium* subsp. *paratuberculosis.* Collins et al (9) identified a repetitive element IS*900* (16, 26) which was used as a probe to generate restriction fragment length polymorphism (RFLP) patterns from isolates of *M. avium* subsp. *paratuberculosis*. Examination of 46 isolates with this method and restriction endonuclease analysis (REA) led to the definition of two groups of isolates with different animal host distributions, broadly defined as cattle [C] and sheep [S] strains (10). There were also phenotypic differences between S and C strains, the former being very difficult to cultivate in vitro. Strains with an intermediate [I] RFLP pattern most similar to S strains were identified in a subsequent study (14). This simple classification of *M. avium* subsp. *paratuberculosis* was confirmed in an independent study (3). RFLP typing data are now available for many hundreds of isolates from widely-separated geographic regions but principally from the northern hemisphere (30) (Table 1). Almost without exception and regardless of geographic location, isolates from cattle have been of C type, as have most isolates from goats and deer. In complete contrast, isolates from sheep have been of C, S or I type, with most countries tending to have only one type in their sheep population. The under-representation of S strains in cattle, deer and goats might reflect the difficulty of laboratory culture of such strains, but their identification in sheep from a similar range of countries suggests that culturability alone does not explain the apparent segregation within host species. Indeed, a C strain was recovered from a cow and an S strain from 7 sheep on the same farm in one study (3). That isolation of *M. avium* subsp. *paratuberculosis* from cattle with paratuberculosis is rarely reported to be difficult is indirect evidence that S strains are uncommon in cattle. Although a considerable amount of information is being compiled in Europe (30), little information is available to assess whether there have been patterns of transmission of paratuberculosis within and between species and regions. A recent report suggests that there may be significant differences in the prevalence of particular RFLP types in cattle with paratuberculosis in Europe compared to South America (25).

Table 1. Reported occurrence in ruminants of strains of *M. avium* subsp. *paratuberculosis* where the presence of IS*900* was confirmed.

Country	No. isolates	Restriction enzyme	No. isolates of each RFLP type			Reference
			C	S	I	
Isolates from cattle						
New Zealand	13,2	*Bst* EII, others	13,2			(10, 12)
Australia	6,5,47	*Bst* EII, *Pst* I, others	6,5,47			(6, 10, 12)[a]
S. America	50	*Bst* EII, *Pst* I	50			(25)
N. America	29	*Bst* EII, others	29			(43)
USA	1	*Pvu* II	1			(40)
Denmark	5,3	*Pvu* II, *Pst* I	5,3			(28, 40)
France	7	*Pst* I	7			(28)
Germany	9,2	*Pvu* II, *Pst* I	9,2			(28, 40)
Hungary	1	*Pst* I	1			(28)
Czech R.	16	*Pst* I	16			(28)
Slovakia	39	*Pst* I	39			(28)
Morocco	1	*Pvu* II	1			(40)
Various	381	*Bst* EII	380	1		(29)[b]
Isolates from sheep						
New Zealand	7,2	*Bst* EII, others		7,2		(10, 12)
Australia	5, 11	*Bst* EII		5,11		(6, 12)
Canada	7	*Bst* EII	6		1	(10)
Faroe	1,1	*Bst* EII, Pvu II		1,1		(10, 40)
N. America	2	*Bst* EII, others	2			(43)
France	1	*Pst* I	1			(28)
Czech R.	14	*Pst* I	14			(28)
Greece	1	*Pvu* II		1		(40)

Country	No. isolates	Restriction enzyme	No. isolates of each RFLP type			Reference
			C	S	I	
Morocco	26	*Pvu* II		26		(3)
S. Africa	5,2,2	*Pvu* II, *Bst* EII, others		2	5,2	(3, 12, 14)
Various	28	*Bst* EII	24	2	2	(29)
Isolates from goats						
New Zealand	9	*Bst* EII	8	1		(10)
Australia	3	*Bst* EII, others	3			(12)
N. America	2	*Bst* EII, others	2			(43)
Norway	1,16	*Bst* EII, *Pvu* II	1,16			(10, 40)
Germany	7	*Pvu* II	7			(3)
Czech R.	1	*Pst* I	1			(28)
Various	17	*Bst* EII	16	1		(29)
Isolates from deer						
New Zealand	20	*Bst* EII	17	3		(13)
S. America	11	*Bst* EII, *Pst* I	11			(25)
Denmark	2	*Pvu* II	2			(40)

[a]All isolates were of *Bst* EII cattle type based on a match between *Pst* I and *Bst* EII genotypic category.

[b]Countries represented were Europe (91.6% of isolates), New Zealand and Australia (5.3%) and USA (3.1%).

1.1.1. Review of literature since November 1999, adding to evidence for cross-species transmissions between sheep and cattle

Follow-up investigations of a trace-back (B2) of one of the 3 Australian farms (B1) reported by Whittington 1998a and VetComm Report 1999 (Appendix 4), detected 2 ELISA reactors (2.1, 2.3) in a serological survey in 1999 (Whittington, Taragel, et al. 2001). These animals had no clinical evidence of disease, but histological examination at post-mortem showed focal granulomatous enteritis with scant or absent acid-fast bacilli. Both were positive on culture for *M paratuberculosis* (S strain). Previous non-clinical ELISA reactors had been detected on B2 in 1993, one of which was confirmed on histopathology, and retrospectively typed as S strain in 1998.

The retrospective analysis of archival formalin-fixed paraffin-embedded tissue samples also identified S strain in paratuberculous cattle and sheep in Iceland, where there had been direct or indirect contact of calves with paratuberculous sheep (Whittington, Taragel, et al. 2001).

Follow-up of Muskens *et al* 1999, showed that *M paratuberculosis* was isolated from 10 sheep from 8 of the 15 flocks which were selected for necropsy (Muskens *et al*, 2001). These flocks had been originally selected as coming from farms with known paratuberculosis infection present in the cattle herd, but no history of paratuberculosis in the sheep. Sampling was done by selecting the 5 ELISA positive animals from the previous survey, and an additional 45 ewes in poorest condition, biased towards farms with the highest percentage of cows with clinical paratuberculosis. Histopathology was positive in all but 2 of the 10 animals, one of which had *M paratuberculosis* isolated only from the tonsil/retropharyngeal lymph nodes. Additionally, all ELISA positive sheep were negative on culture. However, conventional culture only (not BACTEC) was done and strain typing was not reported. Cattle strains are suspected based on the geographic region.

1.1.2. Evidence against cross-species transmission between sheep and cattle

A serological survey of 3 properties in the OJD endemic area (Central Tablelands of NSW) that also ran cattle failed to detect any reactors to the ELISA test (VetComm Report – Appendix 4).

Cousins *et al* (2000) investigated isolates from 42 Australian cattle (5 from NSW), 8 alpaca (0 from NSW), 3 goats (2 from NSW) and 1 rhinoceros and failed to detect S strains in any of these species. However, these isolates were sourced between 1993 and 1996, prior to the regular use of BACTEC culture and ovine media, so the study was biased towards typing of cattle strains.

In summary, "S" strain of *M paratuberculosis* has been isolated from cattle on a number of occasions and in a number of countries. This suggests that although a relatively rare event, it is likely to happen again in circumstances that are favourable for transmission.

2. BACKGROUND AND INDUSTRY CONTEXT

S strain (OJD) was retrospectively diagnosed the late 1990s in 3 cattle from 3 NSW properties (Whittington, Taragel, et al. 2001) where disease had occurred between 1989 and 1995. A presumptive diagnosis was made on another property with clinical JD in a bull, which was sourced from one of the 3 properties. A serological survey detected 2 additional animals on that source property 1999, and these were also confirmed as S strain. Additionally, a small serological survey of at-risk cattle on 3 properties was conducted in 1997, with 426 animals testing negative to the ELISA.

This study builds on the data currently available for evidence of transmission (and lack of it) from sheep to cattle.

Many properties that have had or been at risk for OJD would have run cattle as an additional or alternative enterprise. As part of NOJDP trial 1.1, the use of cattle as an alternative (interim) enterprise was recommended for the destocking period (2 summers) for eradication/control of OJD.

The research described in this report is Phase 2 of a multiphase research program. It builds on projects TR.022 and NOJDP Trial 1.1 and it addresses some of the questions associated with ovine Johne's disease transmission to cattle.

3. PROJECT OBJECTIVES

To address the following by on-farm research:

- To determine the prevalence of disease in cattle exposed to sheep infected with OJD.
- To determine the likely risk factors associated with cattle developing infection or passive transfer of OJD and hence modifications that may need to be applied to OJD control/eradication plans.
- To identify the need for longer term study to examine the persistence of OJD infection in cattle.

4. MATERIALS AND METHODS

4.1. Study design

A prospective survey was conducted on farms selected from NOJDP Trial 1.1 participants (and one other outside that trial) who had susceptible cattle on their property during the time of OJD infection. Trial 1.1 properties were selected on the basis of significant levels of OJD infection. Eligible cattle were defined as having had contact with infected sheep or sheep faeces up to and including age 6 months and were at least 2 years of age at the time of testing. Almost all animals were home-bred.

4.2. Laboratory and other methods

Private veterinary practitioners were recruited to collect serum and faeces from each eligible animal on the property. The sample collection protocol that was followed is included in Appendix 1. This defined animal eligibility and procedures to be followed to ensure best quality samples. Samples were then submitted to RVL Orange, and booked in according to sample receival protocol (Appendix 2).

4.2.1 Serology

Blood samples were collected from either the tail vein or jugular vein using plain Vacutainer® tubes. They were allowed to clot at room temperature and the serum was tested using the bovine Johne's disease absorbed enzyme-linked immunosorbent assay (ELISA) for antibodies against *M.avium* subsp *paratuberculosis*. An ELISA ratio of < 1.5 is regarded as negative, a ratio of > 2 is positive, and between those 2 values is inconclusive in herds where a suspicion of Johne's disease exists.

4.2.2 Faecal culture

Faeces were collected from the rectum of each animal in 70ml sterile containers at the same time as the blood. Matching IDs were placed on the blood and faecal containers, and a separate glove was used for each animal to prevent cross contamination.

Upon receipt at RVL Orange, the samples were either:

- Decanted into pools of 2 into 35 ml sterile tubes, and a single (10 ml) backup sample of each individual sample was placed in storage at –80 ºC, or
- Placed in –18 ºC for 24 hours and then at –80 ºC for storage until staffing availability permitted pooling. Samples were then thawed and pooled as above.

Note: This was a non-standard method for bovine faecal culture preparation.

After pooling, samples were forwarded to RVL Menangle. Samples were again placed in –80 °C storage, if necessary, prior to preparation for culture in liquid radiometric medium as described in Whittington *et al*, 1999a. Growth index (GI) was determined weekly using an automatic ion chamber (BACTEC 460; Johnson Laboratories, Towson, Md.). Samples were collected for PCR and inoculation into modified 7H10 agar when GI was greater than 200. If GI did not reach 200 by 12 weeks then the culture was classified as negative on BACTEC. If significant GI occurred then IS900 PCR (Whittington *et al* 1998b) was performed on samples from liquid culture, and additionally any significant colonies from the solid media. If PCR was negative and no growth occurred on solid media then the culture was classified as negative for *M paratuberculosis*. If PCR was positive from BACTEC liquid culture, but no growth occurred on solid media then the culture was classified as DNA consistent with *M paratuberculosis*, negative on solid media. If PCR of solid media significant colonies was positive, then the culture was classified as positive for isolation of *M paratuberculosis*. If IS900 PCR was positive then IS1311 PCR was done to determine strain type (Whittington, Taragel, et al. 2001). This method enabled the isolation of either S or C strains of *M avium* subsp *paratuberculosis*. See 4.4.2.5 Identification and typing below.

4.2.3 Post -mortem examination

Reactor animals (serology and/or faecal culture) were slaughtered at abattoirs, and samples of intestines and lymph nodes, as per the Cattle MAP guidelines (Anon, 2000), examined and collected, by either the principal research investigator or RLPB District Veterinarians. Tissues collected included proximal ileum, distal ileum, ileocaecal valve, proximal colon, mesenteric lymph nodes (including caudal jejunal) and ileocaecal node.

4.2.4 Tissue culture

Samples collected at post mortem were stored at –80°C until prepared for culture in liquid radiometric medium, as described in Whittington *et al* 1999a. The culture was performed and classified as for faecal culture in 4.4.2.2 above.

4.2.5 Identification and typing

DNA extracted from BACTEC culture and/or solid media was identified using IS900 PCR-REA and IS1311 PCR-REA primers (Marsh *et al* 1999 as cited in (Whittington, Taragel, et al. 2001) to confirm ovine strain. Additionally, culture was attempted on bovine media, with negative results combined with positive results on ovine media, further confirming ovine strain.

4.2.6 Histopathology

Tissues collected at post-mortem were fixed in 10% buffered formalin. They were then embedded in paraffin, sectioned at 5 microns, and stained with haematoxylin and eosin. Additionally they were stained with a method for acid-fast bacilli; samples tested at one laboratory were stained with Ellis' and Zabrowarny's method (Woods and Ellis, 1994) and at the other laboratory with a Ziehl-Neelsen method (Luna, 1968).

5. RESULTS

The host population risk factors for the 12 properties are summarised in table 2; most of these data were collected for NOJDP Trial 1.1.

Table 2 Host Population Risk Factors

Property				Sheep							
Property ID	**Locality**	**District (RLPB)**	**Area - ha**	**Enterprise**	**Number of head**	**Earliest OJD (year)**	**Likely source of infection**	**OJD Diagnosed (year)**	**In Home-Bred**	**Proportion of mortalities**	**Last OJD (month-year)**
Bar	Carcoar	CT	528	Self replacing Merino (SRM)	1900	1975	Introductions[a]	1996	Y	0.06	Nov-98
Oak	Howlong	Hume	2033	SRM	5386	1987	Neighbour	1998	Y	0.04	Oct-99
Pad	Woodstock	CT	243	SRM/Cross bred(XB)	1437	1993	Introduction	1998	Y	0.02	Sept-99
Val	Millthorpe	CT	802	SRM	3790	1989	Introductions	1997	Y	0.08	Mar-00
Bet	Millthorpe	CT	985	XB/Merino (M)	1850	1990	Bull with suspect OJD[b]	1996	N	Low	Jan-00
Bun	Panuara	CT	646	XB/M	2843	1990	Movements to/from "Bet"	1996	N	Low	Dec-99
Gle	Blayney	CT	1422	SRM	5121	1990	Unknown	1994	Y	0.08	Current
Wah	Woodstock	CT	684	SRM	3332	1991	Introductions	1997	Y	0.05	Nov-99
Azi	Blayney	CT	484	Corriedale/M	1569	1993	Introductions	1998	Y	0.01	Oct-99
Kar	Jingellic	Hume	714	SRM	940	1995	Introductions	1998	N	0.05	1999
Kal	Boorowa	Young	225	SRM	2199	Unknown	Unknown	1997	Y	0.02	Dec-99
Nan	Orange	Molong	707	SRM	2234	Unknown	Unknown[c]	1998		Nil	1999

RLPB = Rural Lands Protection Board

CT = Central Tablelands

[a]Goats with OJD in 1996

[b]Single Bull with OJD in 1993

[c]Cattle with BJD diagnosed in 1995

Eight of the 12 properties were from the Central Tablelands RLPB, which has the highest known prevalence of OJD in NSW, being 10% flocks known to be infected compared to total State average of 1.6% (Sergeant, 2001). Most properties could identify that sheep introductions were the most likely source of OJD, and infection had become endemic with disease being diagnosed in homebred sheep. Mortalities in sheep due to OJD ranged from negligible to 8%.

The duration aspects of infection are summarised in figure 1.

Figure 1 Duration of OJD infection on properties

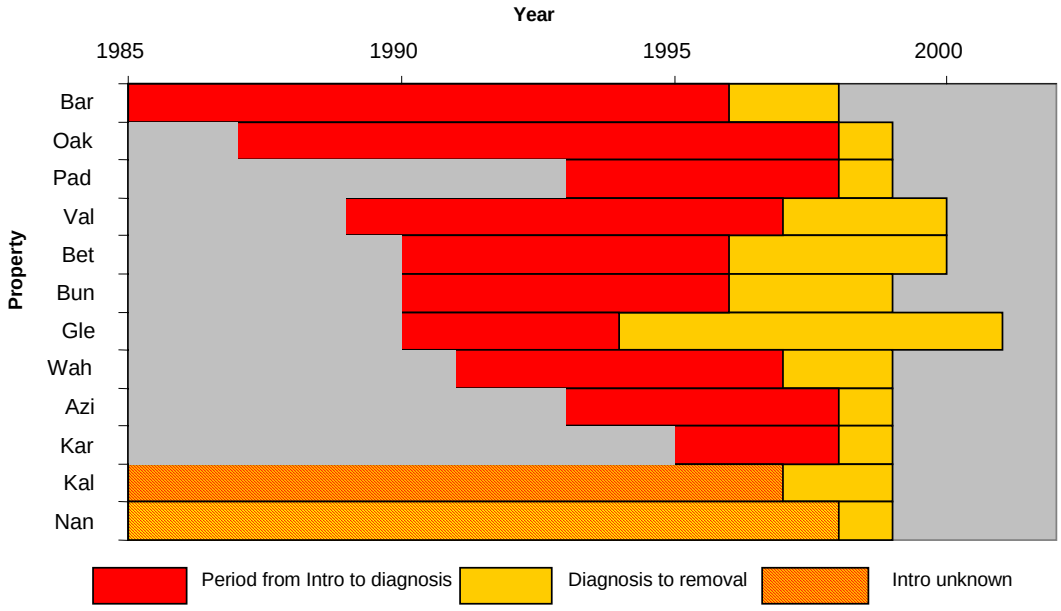

Figure 1 and Table 2 show the shortest time that OJD was recognised as being present on the property was 4 years (Kar) and the longest time was more than 20 years (Bar).

Cattle enterprise details and grazing practices are summarised in table 3.

Table 3 Cattle enterprise and grazing practices

Property ID	Enterprise	Breed(s)	Herd size	History of ill thrift	Grazing practises used - ie sheep with cattle:			
					Co-Grazing	Following	Leading	Shared watering
Bar	Beef	Murray Grey(MG)/ Dexter	214	N	✓		✓	✓
Oak	Beef	AngusX/MG,	509	N	✓	✓		✓
Pad	Beef/Dairy heifers	MG/Friesian	90	N			✓	
Val	Beef	Hereford(Her)/ HerX	73	N			✓	
Bet	Beef	Her/HerX	985	Y[b]			✓	
Bun	Beef	Her/HerX	214	N			✓	
Gle	Beef	Angus	722	N		✓	✓	✓
Wah	Beef	MG/Her	123	N	✓	✓		✓
Azi	Beef	MGX	100	Y[a]	✓			✓
Kar	Beef	Angus/MG	700	N	✓			
Kal	Beef	Shorthorn	123	N	✓			
Nan	Beef stud	Shorthorn	422	Y[c]			✓	

[a] Malnutrition in young animals

[b] Bull with clinical Johne's disease, presumptively typed as S strain because it was sourced from a property with confirmed S strain paratuberculosis in cattle (VetComm Report, Appendix 4).

[c] previous history of BJD, confirmed by culture.

Table 3 shows that history of clinical evidence of Johne's disease in cattle was observed on 2 properties. Total herd sizes (including calves) ranged from 90 to 985 and all were beef enterprises apart from 1 herd which had a portion of dairy heifers. A variety of single or mixed management practices were reported for all properties. Those with least exposure had cattle only grazing ahead of sheep (n=4). Presumably, the highest exposure would have occurred when cattle co-grazed with infected sheep (n=6).

The results of prospective cattle testing done during this study (potentially cross-infected population) are provided in table 4.

Table 4 Cattle sampling and test results

Numbers of animals sampled			Test results (number of animals/pools tested)				
Property ID	**Sera and faeces**	**Pools (of faeces)**	**ELISA Negative Sera**	**BACTEC Negative Pools**	**IS1900 PCR and Solid Media Negative Pools**	**IS1311 PCR Positive Pools**	**Post mortem and Histology negative**
Bar	47	24	47	21	3	-	-
Oak	350	175	350	159	16	-	-
Pad	40	20	40	19	1	-	-
Val	107	54	107	54	-	-	-
Bet	285	143	285	137	6	-	-
Bun	170	85	170	75	10	-	-
Gle	115	58	115	56	2	-	-
Wah	44	22	44	19	3	-	-
Azi	23	12	23	12	-	-	-
Kar	190	94	190	64	22[a]	-	-
Kal	74	37	74	28	9	-	-
Nan	329	329	329	325	3	1[b]	1[b]
Totals	**1774**	**1053**	**1774**	**969**	**75**	**1**	**1**

[a] 8 pools contaminated due to delays in transit.

[b] All followup investigations were negative and herd was given NA status. It is currently testing to progress to MN1.

All herds tested had negative serology. One of the 1774 animals had positive faecal culture, which was typed as S strain. This calculates to an apparent prevalence of 1 in 1774 (=0.06% with 95% CI of 0% to 0.2%). Assuming a 50% to 30% sensitivity of the testing procedure, this gives an upper limit of true prevalence of 0.4% to 0.6%.

6. DISCUSSION

Investigation of these 12 selected properties with known exposure of cattle to OJD resulted in minimal evidence of S strain infection and no evidence of disease being found in cattle. The absence of disease in these properties under study suggests cattle may not readily be infected with exposure to *M paratuberculosis* (S strain). One property investigated in this study (BET) was farm B1 in the previous VetComm report (Appendix 4) and (Whittington, Taragel, et al. 2001). This supports the notion of lack of transmission of OJD within cattle as all animals were serologically and faecal culture negative.

The failure to detect immunological or pathological evidence of OJD infection in 1774 animals from 12 properties suggests that the prevalence is very low. The single faecal culture positive animal with all negative follow-ups may have been carrying the organism passively, or have been in the very early stages of infection.

The chance of cross-contamination occurring in the laboratory was negligible due to the total separation of handling of ovine and bovine samples. Additionally, the success of subculture on solid media negates the possibility of DNA contamination (Graeme Eamens, 2002, pers comm).

Infected sheep had been on the property within the previous 12 months from the date of testing. Research data from trials on the survival of the OJD organism suggest that ingestion of viable organisms and therefore passive shedding could occur for as long as 12 months after destocking (NOJD project OJD.055A). Multiple neighbours are also infected with OJD, which may be a source of ongoing exposure.

The Addendum to this report describes 2 properties where subclinical or early clinical paratuberculosis (S strain) was detected in cattle which were undergoing MAP testing.

6.1. Estimated within herd prevalence of OJD in cattle exposed to OJD

The estimated within herd prevalence of infection with S strain was determined using data from previous investigations (Whittington, Taragel, et al. 2001) and additional investigations which are included in an addendum to this report. These data are summarised in Table 5.

Table 5 Within herd prevalence of known OJD infected cattle herds

Farm ID[a]	Year of diagnosis	Cattle tested	Test results		Prevalence estimates		
			ELISA positive	Histology positive	AP[b]	Avg AP for farm	TP[c] for farm
A	1989	24	-	1	4.2%	4.2%	8.4%
B1	1993	370	7	1	1.9%		
B1	1994	168	2	-	1.2%	1.5%	3.0%
B2	1993	473	21	1	4.4%		
B2	1994	430	9	-	2.1%		
B2	1995	460	0	-	0.0%		
B2	1996	103	1	-	1.0%		
B2	1999	422	2	2	0.5%	1.6%	3.2%
C1	1995	160	-	1	0.6%	0.6%	1.2%
Gil	2001	255	2	2	0.8%	0.8%	1.6%
Ath	2001	183	2	2	1.1%	1.1%	2.2%
Total Farm Prevalence estimates						**1.6%**	**3.2%**

a For explanation of farm ID see appendix 4 (VetComm report) and appendix 2 (addendum to project).

bAP = apparent prevalence, calculated as the number of animals testing positive as a % of the number in the herd at the time.

cTP = true prevalence, crudely estimated as approximately twice AP (Cannon & Garner, 1999). This means that testing of the animals will only find half of those that are actually positive (assuming a test sensitivity of 50%), and close to 100% of those that test positive in a known infected herd are true positives.

However, these prevalence estimates are based on a small number of herds (6), including small herds, and are thus likely to be biased towards a higher prevalence than that expected more generally. It is also likely that these herds are among the highest prevalence herds, so that this estimate of within-herd prevalence of 3.2% is probably an upper limit.

6.2. Estimated herd (area) prevalence of OJD in exposed cattle

The lack of robust data on within-herd prevalence means that it is not possible to reliably estimate the maximum number of cattle herds infected with OJD. What is known at present, however, is that there minimum of 6 cattle herds in NSW with OJD.

One prevalence estimate has been calculated. One animal in 1774 was found to be carrying *M paratuberculosis* without any other evidence of disease. Treating the entire population as having a common within-herd prevalence, the figure of 1 in 1774 suggests an upper confidence limit for probability of an exposed animal becoming infected of around 0.4% to 0.6%. depending on the estimate of the test sensitivity used (Rob Cannon, 2002, pers comm). This estimate is much lower than the observed within-herd prevalences (Table 5), suggesting that there is likely to be significant clustering of infection. ie relatively few herds may be affected, but when transmission does occur it may be at greater levels than the 1 in 1774 suggests. If the probability is 0.4% then about 30% of small exposed herds may have one or more cases, and 65-85% of large exposed herds may have one or more cases. These figures were calculated using a binomial probability distribution (Evan Sergeant, 2002, pers comm). If the risk is lower, (say 0.1% which is about the 50^{th} percentile of the estimate for 1/1774) then these figures drop to 10% for small herds and 30-40% for large herds. Data on herd sizes would have to be sourced from RLPBs to give estimates in absolute terms.

It should be stressed that these estimates are for the population at risk as defined in this study, namely those herds with susceptible cattle exposed to OJD and not the total population of cattle in the study area.

6.3. Risk factors associated with cattle developing infection or passive transfer of OJD

Finding one animal in 1774 to be carrying *M paratuberculosis* without any other evidence of disease suggests that the chances of detecting passive transfer are very low.

Because there were no cases of paratuberculous disease detected in this study, it is not possible to assess risk factors for developing OJD infection. However, some additional information appears in the addendum to this report on factors associated with the 2 additional positive properties.

Johne's Disease has been diagnosed in one herd of cattle in the Goulburn RLPB District. However, OJD is a more recent introduction to that District than the Central Tablelands. The first case of OJD in the Central Tablelands was diagnosed in 1979 (Seaman et al, 1981), whereas it was not diagnosed in Goulburn until 1990. This suggests that the duration of infection on a property may affect the likelihood of cattle picking up infection.

6.4. The need for longer term study

The within herd and herd (area) prevalence estimates from this study need refining. Additional surveillance for OJD in cattle on infected properties could provide this information. Investigation of data from RLPBs in addition to Central Tablelands will give a better estimate of the state-wide picture. Access to RLPB stock numbers data could give a better estimate of the number of exposed susceptible cattle. Privacy issues prevent this at present. A more detailed survey of grazing practices combined with OJD prevalence data could give a better estimation of the level of exposure of cattle to OJD. This would assist in getting a better estimate of State and National level of risk and likely number of infected herds.

Investigation of NSW Agriculture laboratory records for Goulbourn RLPB since 1993 show two instances of cattle JD reactors; one of which was confirmed infected in 1996. BJD Market Assurance Program records from 1996 to 2001 (see addendum) show 3 herds each with 1 reactor have been detected in the Goulburn RLPB. These animals were found not to be infected.

David Kennedy has recently collated national data on cross species infection of cattle with S strain and sheep with C strain (BJD TAG 5, Item 10, July 2002). The conclusion reached in this paper was that

cross infection is occurring at a low rate, but has been reported more frequently than was expected seven years ago when a national approach to JD control began on the assumption that the infections in each species were epidemiologically distinct.

Consideration should be given to funding additional surveillance of cattle on OJD affected farms in the residual and control zones as part of the NOJD program, with contrasts to be made between residual and control zone areas. This may enable better risk classification of cattle on OJD infected properties:

- eg. long duration of property infection with OJD and/or environmental conditions favouring survival of *M paratuberculosis* may pose a greater risk to cattle than properties where OJD has only been present for a short time (and eradicated).

6.5. Additional Validation of the ELISA for suitability as a screening test for CattleMAP

This study showed that for the 1774 animals tested, there was no significant advantage in using faecal culture over the ELISA; all animals were ELISA negative, and the one animal that had one faecal culture positive was negative in all follow-up investigations. The recent BJD TAG (Session 5 item 5) review on the performance and use of absorbed ELISAs for JD in cattle determined that the level of sensitivity of the ELISA test may be affected by herd management factors as well as timing of sampling (age and stage of lactation).

7. SUCCESS IN ACHIEVING OBJECTIVES

- Determining the prevalence of disease in cattle exposed to sheep infected with OJD:

  This was assessed as low, but could not be precisely determined. An estimate would be up to 0.4% to 0.6% of cattle running on OJD infected properties may be infected with *M paratuberculosis* (S strain), based on 1 culture positive out of 1774 animals tested.

- Determining the likely risk factors associated with cattle developing infection or passive transfer of OJD and hence modifications that may need to be applied to OJD control/eradication plans:

  Risk factors could not be determined from this study due to the absence of disease. However, in the properties reported in the addendum, exposure to calves may occur due to run-off from infected neighbouring property(s) or from hand feeding sheep and cattle together during drought.

- Identifying the need for longer term study to examine the persistence of OJD infection in cattle:

  This has been outlined in 4.6.4 above.

Additionally, the following benefit was achieved:

- Support for the value of using the ELISA as a means of cattle MAP screening by comparison with faecal culture results.

8. IMPACT ON MEAT AND LIVESTOCK INDUSTRY

The diagnosis of S strain (OJD) in cattle has now been made on 6 properties in NSW (Whittington, Taragel, et al. 2001) also see Appendix 3). This study builds on the data currently available for evidence of transmission (and lack of it) of OJD from sheep to cattle.

Many properties that have had or been at risk for OJD would have run cattle as an additional or alternative enterprise. As part of NOJDP trial 1.1, the use of cattle as an alternative (interim) enterprise was recommended for the destocking period (2 summers) for eradiation/control of OJD. Only adult cattle should be used for this purpose.

9. CONCLUSIONS AND RECOMMENDATIONS

- The prevalence of paratuberculosis (S strain) in cattle running on OJD infected properties appears to be very low. It is not possible to estimate the maximum number of OJD infected cattle herds but the minimum number is known to be 6 herds.

- There is, however, ongoing evidence of transmission of OJD to cattle (see addendum – Appendix 4).

- S strain infection of cattle may be an emerging problem in OJD endemic areas. At least 6 known properties with autochthonous cases of JD in cattle in the Central Tablelands RLPB District have been confirmed or presumptively diagnosed as S strain. There is evidence of detecting more farms over time. This has been due to more are looking as a result of increased awareness of the possibility of cross species transmission.

- Where cattle are being reared in OJD endemic areas, care should be taken to minimise contact with infected sheep or manure, particularly for at least the first 12 months of life. This would include contact with neighbouring infected properties or exposure to run-off. Care should also be taken to avoid mixing cattle with infected sheep during times of hand feeding.

- Testing of cattle for JD in OJD endemic areas should include culture for OJD if BJD culture is negative in the case of serological reactors.

- Cattle MAP guidelines should include the possibility that OJD will transmit to cattle, particularly in endemic areas.

- It was not possible to determine if cattle can act as reservoirs of OJD to reinfect sheep from this study. However, the presence of clinical and subclinical disease in cattle infected with *M paratuberculosis* (S strain) from previous studies and the addendum to this report indicates that an infected bovine is likely to be a risk to an OJD free sheep flock.

- The addendum to this study suggests that serological screening of cattle in OJD endemic areas, where cattle have had significant contact with infected sheep, may be necessary as part of an OJD surveillance and control program.

ACKNOWLEDGEMENTS

I would like to thank participating producers and veterinarians for their willing cooperation in collecting samples; veterinary and technical staff at Regional Veterinary Laboratories, Orange and Camden for the work involved with sample preparation and testing; RLPB staff and Tim Jessep for assistance with providing data; Evan Sergeant and Rob Cannon for assistance with data analysis; MLA for their financial support and flexibility with reporting deadlines.

BIBLIOGRAPHY

Anon (2000). *The Cattle MAP: the Australian Johne's Disease Market Assurance Program for Cattle*. Australian Animal Health Council Ltd.

Cannon, RM and Garner, MG (1999) *Quantification and Assurance of Risk in the Cattle Market Assurance Program*. National Office of Animal and Plant Health, Canberra.

Cousins DV, Williams SN, Hope A, *et al*. (2000). DNA fingerprinting of Australian isolates of *Mycobacterium avium* subsp *paratuberculosis* using IS-900 RFLP. *Aust Vet J*;78:184-190.

Fridriksdottir-V; Gunnarsson-E; Sigurdarson-S; Gudmundsdottir-KB; Chiodini-R (2000) Paratuberculosis in Iceland: epidemiology and control measures, past and present. *Veterinary-Microbiology*. 77: 263-267.

Luna LG(1968). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed. McGraw-Hill Book Company, New York, NY.

Muskens, J., Bakker, D. and de Boer, J. (1999). Paratuberculosis infection of sheep on farms with paratuberculosis infected cattle in the Netherlands. Sixth International Colloquium Abstracts. p 35.

Muskens J, Bakker D *et al*. (2001) Paratuberculosis in sheep: its possible role in the epidemiology of paratuberculosis in cattle. *Veterinary Microbiology*;78:101-109.

Seaman-JT; Gardner-IA; Dent-CHR (1981). Johne's Disease in sheep, *Australian-Veterinary-Journal*., 57: 2, 102-103.

Sergeant ESG (2001). Epidemiological assessment of ovine Johne's disease in New South Wales, Report prepared for NSW Agriculture February 2001.

Whittington, R.J. (1998a). Project TR.022. DNA typing of Johne's disease organisms. Final Report. NSW Agriculture, Camden, NSW. 1998.

Whittington RJ, Marsh I, Turner MJ, et al. (1998b) Rapid detection of *Mycobacterium paratuberculosis* in clinical samples from ruminants and in spiked environmental samples by modified BACTEC 12B radiometric culture and direct confirmation by IS900 PCR. *J Clin Microbiol*;36:701-707.

Whittington RJ, Marsh I, McAllister S, et al. (1999a). Evaluation of modified BACTEC 12B radiometric medium and solid media for the culture of *Mycobacterium avium* subsp*. paratuberculosis* from sheep. *J Clin Microbiol*;37:1077-1083.

Whittington RJ, Reddacliff L, Marsh I, et al. (1999b) Detection of *Mycobacterium avium* subsp. *paratuberculosis* in formalin-fixed paraffin-embedded intestinal tissue by IS900 polymerase chain reaction. *Aust Vet J*;77:392-397.

Whittington RJ, Hope AF, Marshall DJ, et al.(2000) Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis*. IS900 restriction fragment length polymorphism analyses of isolates from animals and a human in Australia. *J Clin Microbiol;*38:3240-3248.

Whittington RJ, Taragel CA *et al* (2001). Molecular epidemiological confirmation and circumstances of sheep (S) strains of *Mycobacterium avium* subsp. *paratuberculosis* in cases of paratuberculosis in cattle in Australia and sheep and cattle in Iceland. *Veterinary Microbiology*; 79:311-322.

Woods AE and Ellis RC (eds). *Laboratory Histopathology: a complete reference*. Churchill Livingston, Edinburgh, 1994.