

Final report

Cross species transmission of Ovine Johne's Disease- Phase 1

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Abstract

Johne's disease was investigated in fibre goats on several farms. The disease was caused by sheep [S] strains of *Mycobacterium avium* subsp. *paratuberculosis*. The infection appeared to be less severe than the same infection in sheep in that fewer goats than sheep became infected, and fewer goats than sheep developed obvious signs of the infection. However, infected goats shed the organism in their faeces and therefore were able to spread the infection to other goats and sheep. Therefore inclusion of goats in the control program for ovine Johne's disease is justified. A communication program is recommended to advise producers that ovine Johne's disease in goats may not be obvious and that testing should be undertaken to ensure disease is not present. The impact of ovine Johne's disease on the fibre goat industry is projected not to be great due to the small number of herds likely to be infected.

Executive summary

This project was undertaken to investigate ovine Johne's disease in goats on farms where the infection was acquired from sheep. Prior to this study there was little or no information about the behaviour of the disease in goats, its detection, its mode of spread or the risk it posed to uninfected goats and sheep.

By summarising the available information about ovine Johne's disease in goats, and conducting surveys on several farms, a picture of the disease in fibre goats was developed. Infected goats most often were detected using laboratory tests and not because they had obvious disease and it appeared that the tendency to develop severe disease was less in goats than sheep. In addition, the proportion of goats infected was less than the proportion of sheep infected on two farms where the disease was established. The reasons for the different disease pattern in sheep and goats are uncertain but may include lower doses of the organisms being acquired from the environment by goats due to their browsing behaviour, a relative resistance to infection on the part of goats or a degree of adaptation of *M. avium* subsp. *paratuberculosis* S strain to sheep rather than goats.

The circumstances that resulted in ovine Johne's disease spreading from sheep to goats on two farms appeared to include high stocking rates and prolonged or continuous direct and indirect contact between sheep and goats.

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1. Background and industry context

Each of the livestock industries in Australia has addressed or is addressing a control program for Johne's disease. The reason for this is the need to reduce actual or perceived impacts from this disease, which include production losses and trade restrictions, nationally and internationally. The goat industries in Australia are aware of Johne's disease in dairy breeds and recently came to terms with the occurrence of the disease in the fibre industry. Transmission of ovine strains of the causative bacterium *Mycobacterium avium* subsp. *paratuberculosis* from sheep to goats has been observed on two properties in NSW. It is uncertain whether the infection has become entrenched in the new hosts, or whether continued contact with infected sheep has been required to maintain the infection in the new hosts. Furthermore it is uncertain whether the new hosts are able to transmit the infection back to sheep. The prevalence of infection in fibre goats has not been determined, nor the means by which they acquired infection. There have also been suggestions from the goat industry that goats are a dead-end host for ovine strains of *M. avium* subsp. *paratuberculosis*. It is important to determine whether ovine Johne's disease in goats is a deadend infection or whether it can be transmitted to in-contact goats and back to sheep. Observations by industry that transmission from infected goats has not occurred may be due to incomplete evidence or too little time having elapsed to see an effect.

A potential benefit of this work to industry will be acceptance or otherwise (according to the results) that goats and sheep are equivalent with respect to regulation of ovine and caprine Johne's disease. An additional benefit will be information on the magnitude of the infection problem in an endemically infected fibre goat herd. Assessment of this information will enable more meaningful regulatory decisions to be made, for example in relation to permitted movements of infected goats and zoning for goats. When undergoing a disease control or eradication program for one species it is important to know how important other species are in the transmission of disease.

The research described in this report is Phase I of a multiphase research program and it addresses some of the questions associated with ovine Johne's disease transmission to goats. To assist all parties with an interest in this subject, the report includes information on related work in goats that predated the establishment of this research project. Other phases of the on-farm research program will address transmission of ovine Johne's disease to cattle and wildlife. In later experimental phases, co-habitation experiments may be conducted to evaluate the potential for cross-species transmission under controlled conditions.

2. Objectives

To address the following by on-farm research:

1. To determine whether transmission of ovine Johne's disease has occurred from introduced goats to healthy homebred goats.
2. To determine the prevalence of ovine Johne's disease in goats on an endemically infected property.
3. To evaluate the occurrence of goats with faecal excretion of ovine *Mycobacterium avium* subsp. *paratuberculosis*.

4. To provide a source of material for validation of culture tests for ovine Johne's disease in goats.
5. To determine whether transmission of ovine Johne's disease has occurred from introduced goats to sheep.

3. Introduction

Johne's disease is a chronic enteropathy caused by *Mycobacterium avium* subsp. *paratuberculosis*. The disease is endemic in sheep and cattle in New South Wales, Victoria, Tasmania and South Australia as well as in most other countries where animal health surveillance has been undertaken. Other species of farm livestock are susceptible to *M. avium* subsp. *paratuberculosis* and in Australia this infection has been recognised also in goats, alpaca, llama and deer. It has also occurred in a rhinoceros in a zoological collection.

Johne's disease was reported in goats in Australia in 1977¹ but until 1995 cases were confined to dairy breeds and enterprises. In 1995 a probable case of ovine Johne's disease was detected in a cashmere goat in New South Wales. Tracing investigations led to the detection of ovine Johne's disease in both sheep and cashmere goats on a property from which goats had been sold to several other farms. In 1997 ovine Johne's disease was detected in a cashmere goat on a second property in New South Wales unrelated to the initial source property. This report is a summary of the investigations conducted to date on the source property and other farms.

M. avium subsp. *paratuberculosis* is a species consisting of several variants. Using Southern blotting with IS900 as probe in restriction fragment length polymorphism (RFLP) analysis, variants have been classified broadly as sheep [S] and cattle [C] strains based on the host of origin². Extensive surveys in Australia have resulted in confirmation that Johne's disease in sheep has been due to infection with S strains of *M. avium* subsp. *paratuberculosis*^{3,4}. There is only one exception, where sheep on a farm on mainland South Australia were infected with a C strain. In contrast, Johne's disease in dairy goats and cattle has almost exclusively been caused by C strains. Data from many other countries supports a view that C strains tend to occur in many species of livestock, whereas S strains tend to associate mainly with sheep³. The infections in cashmere goats detected in New South Wales in 1995 and thereafter were shown to be due to infection with S strains of *M. avium* subsp. *paratuberculosis* and the disease in these goats is therefore called ovine Johne's disease.

Control programs for ovine Johne's disease have been or are being developed in Australia to restrict the spread of the disease and to permit early resumption of commercial activities on affected farms after infected stock have been removed. It is vital that such programmes properly consider all susceptible or potentially susceptible species of farm livestock and the risk associated with these animals to the control of ovine Johne's disease.

4. Materials and methods

MLA is committed to investing in top quality scientific research, performed by suitably qualified, experienced and registered researchers and organisations. In experiments that involve livestock, MLA acknowledges that such research will first need to be assessed, and if deemed relevant, approved by a recognised Animal Care and Ethics Committee (AEC). The responsibility for obtaining AEC approval lies with the researcher. MLA has in the past not specifically asked for evidence that such AEC approval had indeed been obtained.

4.1 Study design

The results consist of a summary of data from existing field and laboratory investigations as well as additional data from cross-sectional or prospective surveys conducted on three farms under the auspices of the National Ovine Johne's Disease Control and Evaluation Program.

Farms were identified where Johne's disease in goats may occur due to S strain *M. avium* subsp. *paratuberculosis* infection, where sufficient time may have elapsed (> 2 years) for spread amongst goats to have occurred and where the owners were cooperative. Samples were collected for ELISA and/or gel test and faecal culture from animals greater than 2-year-old. Opportunistic evaluation of tissues from animals sent for slaughter was also undertaken if they were thought to have been at a high risk of infection.

4.2 Laboratory and other methods

4.2.1 Serology

Blood samples were collected from the jugular vein, allowed to clot at room temperature and serum was tested in duplicate using the caprine Johne's disease absorbed enzyme-linked immunosorbent assay (ELISA) for antibodies against *M. avium* subsp. *paratuberculosis*⁵. An ELISA ratio > 2 is regarded as positive while a ratio >1.5, <2 is regarded as inconclusive.

4.2.2 Histopathology

Intestinal tissues and associated lymph nodes were fixed in 10% buffered neutral formalin, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin and by a Ziehl Neelsen method⁶.

4.2.3 Culture

Prior to 1998 tissues and faeces were cultured using Herrold's egg yolk medium (HEYM). After 1998 faecal samples and samples of intestine and mesenteric lymph nodes were cultured in a radiometric system (BACTEC) and on modified 7H10 agar^{7,8}. The latter method enables isolation of both S and C strains of *M. avium* subsp. *paratuberculosis* whereas the HEYM method is suitable only for C strains.

Identification and strain typing of *M. avium* subsp. *paratuberculosis* - DNA was extracted from 5 µm sections cut from formalin-fixed paraffin-embedded tissues and *M. avium* subsp. *paratuberculosis* was identified using IS900 PCR-REA (primers 150C/921) and IS1311 PCR-REA (primers M56/M94) as previously described^{9,10}. Identification and strain typing of isolates of *M. avium* subsp. *paratuberculosis* from BACTEC radiometric medium was achieved using the same approach but with different primer sets (P90/P91 and M56/M119).

Alternatively, *M. avium* subsp. *paratuberculosis* was purified from scrapings of the intestinal mucosa of animals with Johne's disease, DNA was extracted and purified and strain typing was undertaken using restriction fragment length polymorphism (RFLP) analysis using *Bst* EII and an IS900 probe as previously described^{3,11}

Table 1. Summary of history of cases of ovine Johne's disease in goats. Where there were no clinical signs of Johne's disease the diagnosis was obtained after application of laboratory tests during tracing or surveillance investigations.

Farm and case number if multiple cases	Location	Origin	Year moved	Year diagnosed	Year of birth	Age at diagnosis (years)	Clinical signs	Contact as kids with infected sheep	Serology	Culture faeces	Culture tissues	Histopath.	Faecal smear	Comment
1. 1	Cmangrove	Farm 2	1995	1995	1991	5	Yes	Yes	pos	neg (HEYM)	nt	nt	pos	unconfirmed case
2. 1 (955)	Carcoar	Homebred	na	1996	1991 or 1992	4 or 5	Low CS	Yes	pos	pos	pos	pos, mb	pos	
2. 2 (092)	Carcoar	Homebred	na	1996	1991	5	No	Yes	pos	pos	pos	neg	neg	
2. 3 (211)	Carcoar	Homebred	na	1996	1991	5	No	Yes	pos	pos	pos	pos, mb	pos	
2. 4 (Y143)	Carcoar	Homebred	na	1996	1994	2	No	Yes	pos	pos	pos	neg	neg	
2. 5 (153)	Carcoar	Homebred	na	1996	1994	2	No	Yes	pos	neg	neg	neg	neg	unconfirmed case
2. 6	Carcoar	Homebred	na	1999	1994	5	No	Yes	pos	neg	nt	nt	nt	unconfirmed case
3. 1	Neville	Homebred	na	1997	1994	3	No	Yes	pos	pos	nt	pos, mb	nt	
4. 1	Tooma	Farm 2	1996	1998	1989-93	>5	No	Yes	pos	nt	pos	pos, pb	nt	
4. 2	Tooma	Farm 2	1996	1998	1989-93	>5	Low CS	Yes	pos	nt	pos	pos, mb	nt	
4. 3	Tooma	Farm 2	1996	1998	1989-93	>5	No	Yes	pos	nt	pos	neg	nt	
5. 1	Corowa	Farm 2	1995	1999	1993	6	No	Yes	pos	pos	pos	pos, mb	nt	
5. 2	Corowa	Homebred	na	1999	1997	2	No	No	neg	neg	pos	neg	nt	contact with goat case 5. 1
5. 3	Corowa	Homebred	na	1999	1994	5	No	No	neg	pos	neg	pos. pb	nt	contact with goat case 5. 1
C3	Cooma	Farm 2	1996	1997	1987	10	No	Yes	pos	nt	pos	pos, mb	nt	

na - not applicable nt - not tested mb - multibacillary lesion pb - paucibacillary lesion inconcl - inconclusive lesion pos - positive neg - negative low CS - body condition score 1

5. Results

The findings in each case of ovine Johne's disease detected in goats in this study are provided in table 1.

5.1 First case and trace-back to farm 2

Johne's disease was suspected in 1995 on farm 1 located at Central Mangrove in a 5-year-old, male cashmere goat that was in poor body condition with scouring unresponsive to anthelmintics. The goat was seropositive in the caprine Johne's disease absorbed ELISA (ratio 3.81), had acid fast bacilli in a faecal smear but was culture negative for *M. avium* subsp. *paratuberculosis* using HEYM. Unfortunately this goat died and was not submitted for post mortem examination. Nevertheless it is believed to be the first case. The male goat was born in 1991 and had been purchased with two others in February 1995 from farm 2. Infection with an S strain of *M. avium* subsp. *paratuberculosis* was suspected because of the negative culture outcome. This is an unusual result, as typically in Australia goats were known to be infected with cultivable C strains.

5.2 Investigation on farm 2

Farm 2, located at Carcoar in the central tablelands district of NSW consists of 540 Ha of improved and unimproved pasture with a component of broad-leaf weeds. The enterprise comprised self replacing fine wool Merino sheep and cashmere goat breeding, with rotational grazing of sheep and goats to effect weed control. Concurrent grazing of sheep and goats in the same paddock was often undertaken (direct contact) although goats and sheep were also maintained as separate mobs that were moved from paddock to paddock in a cycle but without spelling pasture (indirect contact). During drought periods sheep and goats sequentially were hand fed on the ground in the same area. Stocking rates ranged from 7 sheep and 5 goats per Ha to 5 sheep and 3 goats per Ha between 1984 and 1997. Johne's disease was diagnosed in sheep in March 1996 after trace-back from the goat detected on farm 1 (Table 2). Ovine Johne's disease was thought to have been introduced onto farm 2 after 1982 with purchases of rams from a property now known to have sheep with ovine Johne's disease. The goats were obtained between 1980 and 1983 from the western division of

NSW, a low risk area for Johne's disease, and surplus homebred goats were later sold to other farms.

Following the initial diagnosis of ovine Johne's disease in sheep, serological surveys were undertaken in September and October 1996 to check the goat herd for evidence of Johne's disease and to determine the prevalence of Johne's disease in the sheep flock (Table 2). Seropositive goats (n=5) and sheep (n=11) were submitted for post mortem examination in November 1996. Johne's disease was confirmed in a total of 4 goats by histopathology (2 goats positive) and by culture of mesenteric lymph node (3 goats positive) and faeces (4 goats positive) (Tables 1, 2). The histological lesions in two goats were those of severe, generalised, granulomatous enteritis affecting ileum, caecum and colon; epithelioid cells in the affected tissues contained masses of acid fast bacilli (severe multibacillary lesions). Johne's disease was confirmed in 4 sheep by histopathology (2 with multifocal paucibacillary lesions, 1 with generalised multibacillary lesions) and culture of mesenteric lymph node (4 sheep positive) and

faeces (2 sheep positive). *M. avium* subsp. *paratuberculosis* type S1 was confirmed in 5 sheep and 2 goats from bacteria purified from intestinal mucosal scrapings. S strain was confirmed in both these goats and 1 of the sheep by examining tissue blocks.

All sheep were removed from the farm between March and November 1996. By 1999 the farmer had almost completed a property disease eradication plan and the last remaining goats were due to be removed in November 1999. There were approximately 200 2-to-5-year-old cashmere goats available for examination. Approximately 42 had potentially been in contact as kids with infected sheep but the remainder were either too young to have had direct contact with sheep or were kept separate from sheep between March and November 1996. All goats > 2-year-old were tested by ELISA and individual faecal culture in August 1999 with negative results apart from a single ELISA reactor (5-year-old male, born in 1994) which was lost to follow-up. These results suggest a low prevalence of Johne's disease in the goats at this time.

In 1996 the seroprevalence of Johne's disease in sheep was 4.01% but some of these animals were not confirmed as infected by other tests. The seroprevalence in goats was lower (1.45%). Although the seroprevalences in sheep and goats were significantly different (Chi square 5.50, P=0.02) it is impossible to know whether the true prevalences of infection were different. Nevertheless it would appear that the apparent higher prevalence in sheep was accompanied by a higher rate of mortality attributable to Johne's disease than observed in goats.

Table 2. Results of laboratory tests for farm 2

Date tested	Group	Tests and results	Interpretation/comments
March 1996 ON96/1015	3.5 to 6-year-old sheep (n=2)	Multibacillary Johne's disease lesions in ileum of both ewes.	OJD confirmed in home-bred sheep - endemic infection suspected
September 1996 ON96/2926 MN96/C917	Mature cashmere goats of both sexes (n=482)	7 sera positive (ratios 2.42 to 3.81, range), 5 sera inconclusive (1.63 - 1.83, range) in ELISA, 1 serum positive in gel test. Johne's disease confirmed in 4 of 5 seropositive goats by histopathology and culture after necropsies in November 1996. S strain <i>M. avium</i> subsp. <i>paratuberculosis</i> confirmed in 3 of 3 tissue blocks from one goat and 1 of 3 tissue blocks from another with multibacillary lesions. Strain S1 confirmed in both these goats by testing bacilli purified from the gut wall.	OJD confirmed in home-bred goats. Seroprevalence 1.45% (95% C.L. 0.59-2.97%) Owner did not see signs of Johne's disease in goats and estimated <1% losses overall. Direct and indirect contact of goats with paratuberculous sheep
October 1996 ON96/3189 MN96/C917	Mature Merino ewes (n=374)	15 sera positive in ELISA (cut-off 2.4) with 8 of the 15 positive in the gel test. Johne's disease confirmed in 4 of 11 ELISA reactors by histopathology and culture after necropsies in November 1996. S strain <i>M. avium</i> subsp. <i>paratuberculosis</i> confirmed in 2 of 2 tissue blocks from a ewe with multibacillary lesions. Strain S1 confirmed in 5 sheep with multibacillary lesions by testing bacilli purified from the gut wall.	Seroprevalence 4.01% (95% C.L. 2.26-6.53%) Owner recognised signs of OJD as weight loss and death in mature sheep and estimated losses due to OJD in adult sheep (3-to-8-year-old) at 6% per annum.
February 1997 CM97/339	>2-year-old cashmere does (n=60)	Individual faecal samples culture negative.	Johne's disease not detected
August 1999 ON99/3129 ON99/3149	2-5-year-old cashmere goats (n=200)	1 serum positive (ratio 2.21), 1 serum inconclusive (ratio 1.57) in ELISA (both 5-year-old males). All 200 individual faeces culture negative.	Seroprevalence 0.5% (95% C.L. 0.01 - 2.75%). Johne's disease was not confirmed as the seroreactor was lost to follow-up.

5.3 Investigation on farm 3

Farm 3 consists of 294 Ha of improved pasture located in the central tablelands district of NSW. The enterprise was mixed grazing of sheep and cashmere goats. The goats and sheep were grazed

separately, though there was rotational grazing of sheep and goats in the same paddocks to effect weed control. Does were joined at 12 months of age and kids were run separately from their dams. The stocking rate between 1993 and 1997 ranged between 6 sheep and 1.2 goats per Ha and 7 sheep and 1.3 goats per Ha.

Ovine Johne's disease was diagnosed in homebred Merino sheep on farm 3 in September, 1995 (Table 3). The levels of sheep losses due to ovine Johne's disease was estimated by the owner to be <1%, though the prevalence of seropositive (gel test) adult sheep in November 1996 was 5.8%. The source of infection could not be identified, suggesting non-recent, endemic infection. There is water inflow from an adjacent farm that has a flock with a high prevalence of ovine Johne's disease (also suspected to be the source flock for infection of farm 2); this farm is separated from farm 3 by a double fenced road.

There was no suspected clinical Johne's disease in the cashmere goats. All goats over 2-year-old were tested using the ELISA in August 1996 (n = 51) with negative results and again in September 1997 (n = 46) when a 3-year-old doe (born September, 1994, seronegative in 1996) was positive. A faecal sample taken from this doe was culture positive for *M. avium* subsp. *paratuberculosis*. At necropsy in November 1997 the doe had enlarged and oedematous ileocaecal and caudal mesenteric lymph nodes with mild cording of the lymphatics. Histologically it had multibacillary paratuberculosis lesions in small intestine, caecum, colon and lymph node. Culture was not undertaken. S strain *M. avium* subsp. *paratuberculosis* was confirmed in 1 of 2 formalin-fixed paraffin-embedded tissues.

All adult sheep were sold from the property in March 1997 and were replaced by Merino ewes from Walgett within a few months. All offspring of the original sheep were sold by July 1997. The goat herd was disbanded in August 1998.

Table 3. Results of laboratory tests for farm 3

Date tested	Group	Tests and results	Interpretation
September 1995 ON95/2406	Adult sheep (n=1)	Clinically affected sheep with severe multibacillary lesions confirmed to be <i>M. avium</i> subsp. <i>paratuberculosis</i> S strain in 4 of 4 tissue blocks.	OJD confirmed in home-bred sheep. Endemic infection suspected.
August 1996 ON96/2610	>2-year-old Cashmere goats (n=51)	All sera negative in ELISA	Johne's disease not detected in the goat herd.
November 1996 ON96/4002	Adult Merino ewes (n=294)	17 positive in gel test	Seroprevalence 5.8% (95% C.L. 3.4 - 9.1%)
September 1997 ON97/3380 ON97/3544 November 1997 ON97/4402	>2-year-old cashmere goats (n=46)	1 serum positive in ELISA (ratio 4.16) but negative in gel test. Infection confirmed by faecal culture of <i>M. avium</i> subsp. <i>paratuberculosis</i> . At necropsy in November 1997 the goat had an ELISA ratio of 5.03 and there were gross lesions of JD. Histologically there was granulomatous enteritis, colitis, typhlitis and lymphadenitis with small to moderate numbers of acid fast bacilli which were identified as <i>M. avium</i> subsp. <i>paratuberculosis</i> S strain in 1 of 2 tissue blocks.	Seroprevalence 2.17% (95% C.L. 0.06 - 11.5%) OJD confirmed in seropositive 3-year-old homebred doe which had indirect contact with OJD infected sheep by grazing contaminated pastures.

5.4 Investigation on farm 4

Farm 4 consists of 537 Ha of mostly unimproved pasture at the top of a valley adjoining bushland near Tooma on the southern tablelands of New South Wales. The enterprise on this farm was mixed grazing of Boer goats, cattle and small numbers of sheep. The neighbouring farms had not grazed sheep since 1995, and prior to that not on paddocks adjoining farm 4.

In March 1996, 280 3-to-7-year-old cashmere does (born 1989-1993) were introduced from farm 2. In June 1996 a small number of cashmere does were introduced from a farm at Sale, Victoria.

The introduced cashmere goats were joined with Boer goats and were grazed all over the farm, although when kidding were grazed on 15 Ha of improved pasture, where 20 sheep (homebred and introduced) and 49 Boer goats (homebred and introduced from South Australia) exclusively grazed. During kidding, approximately 400 does grazed this small area and had direct contact with the sheep and Boer goats. The homebred sheep were progeny of ewes that were introduced onto the property as hoggets in December 1993 from a farm at Coolamon, New South Wales. This farm has undergone serological testing (n=507) for ovine paratuberculosis using the gel test between October 1996 and September 1998 with negative results.

In August 1998, cashmere and cross bred does (n=259) were tested using the gel test as a trace forward from farm 2 (Table 4). Most of these cashmere does originated from farm 2, although a small number were from the Sale farm. There were 3 clinically normal serological reactors and each had originated from farm 2. They were 5-to-9-years-old (1989-1993 drop), with body condition scores of 3, 1 and 2. The former two had gross and microscopic lesions of Johne's disease while the latter had no lesions. All 3 goats were culture positive from terminal ileum and typing indicated S strain. At the time of this diagnosis the infected cashmere does had grazed in direct contact with 2-to-6-year-old stud Boer goats (n=49), 1-to-2-year-old first cross goats (n=540) which included progeny of the infected does and 3-to-7-year-old cross bred ewes (n=20). In addition, there were 6-to-10-year-old cows (n=120) from several locations in New South Wales and Victoria. Stocking rates in total were up to 27 head per Ha.

In December 1998, 2-year-old cashmere does (n=21) and crossbred bucks (n=17) (progeny of the introduced farm 2 goats) and mature bucks (n=4) were investigated. These 42 goats were serologically (ELISA), histologically and tissue culture negative for Johne's disease. In June 1999, 2-year-old crossbred does (n=170) which were progeny of does from farm 2 and 2-to-6-year old Boer does (n=28) were negative in pooled faecal culture.

In September 1999, the 20 crossbred ewes of mixed ages (3-to-7-year-old, average 4-year-old) were investigated. The youngest sheep were born in 1996. These 20 sheep were negative on serology (gel test) and histopathology. However, culture of terminal ileum collected from each sheep at an abattoir using instruments cleaned in boiling water and a clean work area for each animal yielded *M. avium* subsp. *paratuberculosis* from 5 sheep. Of the 5 culture positive sheep, 4 were in sequential order. As contamination of samples collected in the abattoir may have occurred, there may have been only one infected sheep. Adequate precautions were taken in the abattoir to prevent major cross contamination so it is likely that more than 1 sheep was actually infected.

The results of laboratory examination of goats from this property suggest that *M. avium* subsp. *paratuberculosis* infection was present in 3 does introduced from farm 2 and may have spread to up to 5 sheep on the farm, but these were at a relatively early stage of the infection and lacked histological lesions (Tables 1, 4). There was no evidence of transmission to home-bred goats but the reasons for this may include too little time having elapsed between introduction of infection and insufficient exposure. In addition, only young adult (2-year-old) animals were tested in the period Dec 1998 to June 1999. These animals may have been too young to test positive, i.e. insufficient time had elapsed for the infection to have developed sufficiently in these animals to be detected. None of the goats or sheep had clinical signs of Johne's disease, except possibly for the 2 introduced culture positive does in 1998 which were in light body condition. The owner had

not noticed any clinical disease or mortalities attributable to Johne's disease in either sheep or goats.

Table 4. Results of laboratory examination of goats and sheep from farm 4

Date tested	Group	Tests and results	Interpretation
August 1998 MN98/9792MN 98/9794 MN98/A169	>3-year- old cashmere and crossbred does (n=259), most of which came from farm 2	3 positive in gel test and <i>M. avium</i> subsp. <i>paratuberculosis</i> isolated from terminal ileum of all three. Two had paratuberculous lesions grossly and histologically (1 multibacillary, 1 paucibacillary). All 3 were from farm 2. S strain <i>M. avium</i> subsp. <i>paratuberculosis</i> confirmed in 2 of 2 tissue blocks from the goat with multibacillary lesions.	JD confirmed in does introduced from an infected farm 2.5 years after their introduction. No evidence of spread to home-bred does.
December 1998 MN98/E844	2-year-old cashmere does (n=21) and cross bred bucks (n=17) (progeny of does from farm 2). Mature Boer bucks (n=4).	All negative in gel test, absorbed ELISA and histopathology of terminal ileum, mesenteric lymph nodes and caecum. Culture negative from tissues.	JD not detected.
June 1999 MN99/8308	2-year-old crossbred does (n=170) and 2-to-6-year-old Boer does (n=28)	Negative in pooled faecal culture.	JD not detected.
August 1999 MN99/A164	3-to-7-year-old sheep (n=20)	Abattoir sampling. All negative in gel test and histopathology of terminal ileum and mesenteric lymph node. <i>M. avium</i> subsp. <i>paratuberculosis</i> S strain isolated from the terminal ileum of 5 sheep.	<i>M. avium</i> subsp. <i>paratuberculosis</i> infection detected in up to 5 sheep. Transmission of infection from goats to homebred sheep confirmed.

5.5 Investigation on farm 5

The property consists of 5 Ha of pasture at Corowa in the Riverina region of New South Wales on which 210 goats, including about 110 > 2-year-old and 5 aged wether sheep were kept by hand feeding on the ground and in feed troughs. In May 1995 a 2-year-old cashmere buck (born 1993) was introduced from farm 2. Due to trace forward investigation from farm 2 this animal was investigated and found to have Johne's disease in May 1999 (Tables 1, 5). This buck had grazed over the entire farm but was often confined with 5-year-old (born 1994) homebred bucks (n=4) on a 0.25 Ha paddock. In August 1999, all goats and sheep over 2-year-old were sampled individually (n=102). All were negative in the gel test and 2 goats were inconclusive in the ELISA. One of the 4 homebred bucks mentioned above was faecal culture positive for *M. avium* subsp. *paratuberculosis*. In September 1999 two 2-year-old bucks and the four 5-year-old bucks mentioned above were necropsied and samples were collected for histopathology and tissue culture. Multifocal granulomatous lesions containing a few acid fast bacilli were present in the lymph node of one goat, while 3 others had granulomas without acid fast bacilli in the intestine or lymph node and two had no microscopic lesions. *M. avium* subsp. *paratuberculosis* S strain was isolated from the terminal ileum of one 2-year-old buck that had focal granulomatous enteritis. Thus overall, two of 6 homebred bucks were confirmed as infected, one by histopathology and faecal culture and one by tissue culture. These observations confirm the transmission of infection between introduced and homebred goats.

Table 5. Results of laboratory tests for farm 5, Corowa

Date tested	Group	Tests and results	Interpretation
May 1999 MN99/6063	Introduced cashmere buck	Gel test and ELISA positive; multibacillary paratuberculosis histologically; <i>M. avium</i> subsp. <i>paratuberculosis</i> isolated from both terminal ileum and faeces	Johne's disease confirmed in a buck introduced from farm 2.
August 1999 MN99/A925	>2-year-old goats and sheep (n=102)	2 sera ELISA inconclusive but gel test negative; 1 individual faecal culture positive for <i>M. avium</i> subsp. <i>paratuberculosis</i> S strain. Sample was from a homebred 5-year-old buck.	Possible infection or passive excretion of organisms remaining on pasture.
September 1999 MN00/2558	2-year-old bucks (n=2) 5-year-old bucks (n=4)	Mild focal to multifocal granulomatous lesions detected in the ileum of 2 goats or lymph nodes of another 2 goats. Acid fast bacilli seen in one lymph node lesion. <i>M. avium</i> subsp. <i>paratuberculosis</i> S strain isolated from terminal ileum of one 2-year-old buck without histological lesions. The buck that previously was faecal culture positive was positive on histopathology.	Infection confirmed in a homebred buck. Spread between introduced and homebred goats confirmed.

5.6 Trace forward investigations in Cooma RLPB

There were three farms in the Cooma RLPB that were known to have purchased goats from farm 2. A trace forward investigation was conducted on each (Table 6).

Farm C1 purchased 2 bucks from farm 2 in 1993. One was present for only one joining period (3 months) and the other for 2 joinings before escaping. The bucks had direct contact with approximately 100 does. The goats were used predominantly for grazing the weed ridden rough country. There was indirect contact of goats with sheep through rotational grazing and use of common facilities. In April 1996, 10 introduced and 10 homebred does were serologically negative (ELISA). In September 1998, 450 sheep that had been in indirect contact with the 2 bucks were serologically negative (gel test).

Farm C2 purchased 2 bucks from farm 2 in 1993 at the same time as property C1. In April 1996 both goats were serologically negative (ELISA and gel) and one buck was autopsied and was histologically negative for Johne's disease. As on farm C1, the goats were used predominantly for weed control. In September 1998, serological testing was undertaken on sheep (n=449) which may have had indirect contact with the goats. There was one sheep with an inconclusive gel test reaction. It was autopsied and was histologically negative.

Farm C3 purchased 9-year-old does (n=3) and one 6-year-old buck in February 1996 from farm 2. In April 1996, all 4 goats were quarantined onto one paddock and were serologically negative (ELISA). The 4 introduced goats co-grazed with other goats for a 2 month period prior to being quarantined by the owner. After that time they remained grazing on a discrete area except when using common animal handling facilities. In April 1997 in a repeat serological test one of the 4 introduced does had a positive ELISA ratio (3.87). Upon further investigation this doe was histologically positive (multibacillary lesions) and tissue culture positive for *M. avium* subsp. *paratuberculosis* (Tables 5, 6). The remaining 3 introduced goats and two 12-month-old offspring of the infected doe were autopsied and were histologically negative. In February 1998, homebred goats (n=35) and sheep (n=10) over 2 year-old were serologically negative. In June 1998, 72 sheep over 2 year-old were serologically (gel) and pooled faecal culture negative.

Table 6. Trace-forward investigations from farm 2 to farms in Cooma RLPB

Farm	Farm 2 goats		Follow-up of homebred goats/sheep
	Movement	Follow-up	
C1. Bungarby	1993: bucks (n=2)	Both escaped, one after the 1st joining, the second after another year	April 1996: does (n=20) ELISA negative (MN96/3595) Sept 1998: sheep (n=450) gel test negative (MN98/A495)
C2. Bobundara	1993: bucks (n=2)	April 1996 both gel and ELISA negative, 1 negative at post mortem (MN96/3594)	October 1998: goats (n=108) gel test and ELISA negative (MN98/B691) Sept 1998: sheep (n=152) gel test negative (MN98/B429) Sept 1998: sheep (n=297) gel test negative, 1 inconclusive in gel test (MN98/A333) with negative histopathology (MN98/F482)
C3. Tharwa	1996: 9-yr-old does (n=3) 6- yr-old buck (n=1)	April 1996: all negative in ELISA (MN96/3734) April 1997: 1 of the does positive in ELISA (MN97/4639) and confirmed infected by histopathology (multibacillary lesions but low numbers of AFB, probably excreting organisms) and intestinal culture of <i>M. avium</i> subsp. <i>paratuberculosis</i> (MN97/5838); remaining 3 animals and 2 1-yr-old kids of the infected doe histologically negative (MN97/D518)	Introduced goats were isolated soon after arrival. Feb 1998 (MN98/2154) >2-year-old goats (n=35) ELISA negative >2-year-old sheep (n=10) gel negative . June 1998 (MN98/6945) >2-year-old sheep (n=72) gel negative and pooled faecal culture negative.

6. Discussion

The suspicion that an ovine strain of *M. avium* subsp. *paratuberculosis* might be the cause of Johne's disease arose in the index case in this study when attempts to culture the organism on Herrold's egg yolk medium (HEYM) were unsuccessful, despite the fact that the animal was seropositive, had advanced clinical Johne's disease and large numbers of acid fast bacilli in a faecal smear. Bovine strains of *M. avium* subsp. *paratuberculosis* are readily isolated on HEYM whereas isolates from sheep with Johne's disease have generally been unsuccessful in Australia using this medium. Technology for culture of S strains and for rapid strain typing from histological material was developed in 1988⁷⁻¹⁰. It was also possible to type the strains of *M. avium* subsp. *paratuberculosis* in the goats after application of methods that were developed in 1996 to purify *M. avium* subsp. *paratuberculosis* from the intestinal mucosa¹¹.

6.1 Features of Johne's disease in goats

6.1.1 Farm 1

Clinical JD occurred in a goat after transport to a new location.

6.1.2 Farm 2

Ovine Johne's disease was transmitted from sheep to goats. This occurred sometime after 1983. There was close contact of goats with sheep, including ground feeding during a drought and goats grazing pasture after being heavily grazed by sheep. Stocking rates were 5-7 sheep per Ha and 3-5 goats per Ha, which are considered high. The prevalence of infection in goats (1.5%) appeared to be lower than in sheep (4%) in 1996 when a serological survey was undertaken. Despite the

apparent low prevalence of infection in goats there were several examples of infected goats detected on other farms after being sold by Farm 2 (see other farms below). The prevalence in goats sourced from farm 2 in 1996 and transferred to farm 4 also appeared to be <2% (see farm 4 below). In addition to differences in the apparent prevalences of infection between goats and sheep, clinical disease in goats was uncommon or absent whereas clinical disease in sheep was very common. Sheep were removed from the farm in 1996. In 1999 there was no evidence of infection in goats that remained on the farm. There was insufficient evidence to conclude whether infection in goats was endemic or whether continued contact with infected sheep was required to maintain the infection in the goat herd.

6.1.3 Farm 4

JD was detected in 3 sheep 2.5 years after movement from farm 2. As with all goats detected after tracing from farm 2, with the exception of the one on farm 1, these goats were not obviously affected with Johne's disease, however 2 were shedding large numbers of organisms in faeces as they were multibacillary cases. The prevalence of infection in 1998 in the herd of goats originating from farm 2 in 1996 seemed to be similar to that in goats remaining on farm 2 in 1996, i.e. about 1.5%. In 1999 the progeny of this infected group of goats appeared to be uninfected. This may have been due to their young age or endemic infection not having yet established on farm 4. However, apparent transmission to sheep was detected in 1999. The sheep had had 3.5 years contact with the infected group of goats, but it is unclear when contact with contaminated faeces commenced because it is uncertain when the goats commenced excretion of the organism. The sheep were 3-to-7-years-old in 1999, and therefore newborn-to-4-years-old when they first came into contact with potentially infected goat faeces, but the ages of the individual sheep were not recorded so it is not possible to determine whether infection was due to lambs being in contact with goats or due to transmission to adult sheep. Given that the goats may not have been excreting organisms when they first arrived on farm 4 there is a possibility that transmission occurred to mature sheep. This may explain the lack of lesions in the sheep and the likelihood that the sheep had silent or latent infection.

6.1.4 Farm 5

There were extraordinarily high stocking rates on this farm and the level of environmental faecal contamination would have been correspondingly very high. Diagnosis of JD in an animal moved from farm 2 in 1995 occurred in 1999. The time of first shedding of organisms was unknown but the infected goat was exposed to a group of 1 year old bucks from the time of arrival. These animals appeared to not contract the infection, and one that was faecal culture positive in 1999 was most likely a passive shedder, having merely consumed organisms then passed them in faeces. However, another goat, that was potentially exposed from birth, had a low grade intestinal infection. As it was only 2 years old when sampled it may have gone on to develop a more serious infection.

6.1.5 Farm 3

The epidemiology of Ovine Johne's disease in goats on this farm appeared to be very similar to farm 2. It was suspected that sheep on farm 3 had been infected for many years. The seroprevalence of infection in sheep in 1996 was 5.8% compared to zero in goats; a very low seroprevalence was detected in goats in 1997. The grazing situation was similar to farm 2 and as on farm 2 there were no clinical cases of JD in goats.

6.1.6 Farm C3

An old infected doe was detected about 1 year after movement from farm 2. Based on histopathology the animal was probably shedding only small numbers of organisms. Mature sheep and goats tested in 1998 were seronegative and faecal culture negative, however insufficient time may have elapsed between exposure and testing for these animals to have developed detectable levels of infection.

6.2 Summary of the pattern of Johne's disease infection in goats

Goats appeared to be more resistant to infection than sheep on the two farms where the infection was endemic. The evidence for this is the fact that on both farms relatively fewer goats than sheep were infected and the disease did not progress to the clinical stage in goats even though mortalities were occurring in sheep. One reason for this pattern of disease in goats is that they may have ingested lower doses of the organism than sheep due to their different grazing behaviour. Another is that they may have greater inherent natural resistance to infection compared to sheep, while still another is that the S strain of *M. avium* subsp. *paratuberculosis* may not be sufficiently adapted to goats to easily or quickly cause clinical disease. In any case the goats on both endemically infected farms had had close contact with infected sheep.

The individual infected goats tended not to be clinical cases. There may be several reasons for this. Firstly, most of the investigations were conducted because of tracing and animals were detected using lab tests in preclinical stages of the disease. The exceptions were the index case on farm 1 which was a clinical case and a possible early clinical case on farm 4, but this animal merely had low body condition score. Six of 12 cases that were examined histologically were multibacillary cases and therefore were excreting organisms in faeces and causing environmental contamination. Several of these animals were examined by faecal smear or culture and were positive, again confirming shedding into the environment.

The infected goats were detected using serological tests for caprine Johne's disease, implying that the strain of *M. avium* subsp. *paratuberculosis* causing the infection is irrelevant for diagnosis.

6.3 Transmission of Johne's disease from infected goats

On the two endemically infected farms (2 and 3) it was not possible to differentiate between infection in goats due to ongoing exposure to infected sheep, or infection due to contact with infected goats. However, on farms TH and K there was evidence of transmission from goats to sheep and goats to goats, respectively. The stocking rates were exceedingly high on both farms [>25 head/Ha on farm 4 and >60 head/Ha on farm 5], a factor that may have favoured transmission, although the proportion of infected goats on both farms was very low.

7. Success in achieving objectives

Objectives 1, 2, 3 and 5 were achieved. Transmission of ovine Johne's disease from introduced goats to healthy homebred goats was demonstrated, the prevalence of ovine Johne's disease in goats on an endemically infected farm was determined, faecal excretion of *M. avium* subsp. *paratuberculosis* in infected goats was confirmed and transmission of ovine Johne's disease from introduced goats to sheep was confirmed.

Objective 4, provision of a source of material for validation of culture tests for ovine Johne's disease in goats, was not achieved. Too few infected goats were detected in the research phase of this project. Most of the infected goats were detected prior to this project commencing and suitable samples were not obtained at that time.

8. Impact on meat and livestock industry

Fibre goats are susceptible to ovine Johne's disease and need to be considered in regulatory action to control ovine Johne's disease. Fibre goat producers need to be aware that ovine Johne's disease may not be obvious in their animals and a communication package needs to be developed to encourage testing. Although testing may reveal additional infected herds, it is likely that tracing investigations from the known source farms are largely complete. Sampling strategies to detect Johne's disease due to S strain *M. avium* subsp. *paratuberculosis* in fibre goats may need to be reviewed due to a lower prevalence of infection and probable lower sensitivity of diagnostic tests in goats compared to sheep.

9. Conclusions and recommendations

Ovine Johne's disease in fibre goats appears to be a relatively mild infection, uncommonly progressing to the clinical stage in animals less than 5 years old. This is in direct contrast to caprine JD in milking goats caused by cattle strains of *M. avium* subsp. *paratuberculosis* which tends to progress to the clinical stage more commonly. The reason for this difference may relate more to husbandry differences between fibre and dairy goats than any real difference in the virulence of the organisms or resistance of the hosts. Dairy goats may be maintained under relatively crowded conditions, tend to be hand fed more often than fibre goats and may be exposed to higher levels of contamination, which would tend to result in more rapid disease progression after infection.

As Johne's disease due to *M. avium* subsp. *paratuberculosis* S strain in goats appears to be a milder infection than in sheep it is reasonable to expect diagnostic tests such as serology and faecal culture to be less sensitive in goats than in sheep. The reason is that these tests are most effective in animals that have proceeded through the early stages of the disease; if fewer animals have done this the tests are less able to detect infection in the herd. Sampling strategies to detect Johne's disease in fibre goats may need to be reviewed to account for lower expected prevalence and lower sensitivity of diagnostic tests in goats compared to sheep.

Goats and sheep need to be considered separately when herds and flocks are sampled for market assurance testing because of differences in the prevalence of infection and likely performance of diagnostic tests between the two species. At the time of writing the sensitivity of diagnostic tests for Johne's disease in fibre goats is uncertain however, faecal culture is generally considered to be more sensitive than serology in other ruminants. Pooled faecal culture has not been validated for use in goats but there is a need to do so to reduce the costs of testing herds to market assurance program standard.

Fibre goats infected with *M. avium* subsp. *paratuberculosis* S strain shed the organism in faeces and are capable of maintaining the infection in a flock/herd situation. Transmission of infection to sheep and goats is clearly possible. For these reasons fibre goats must be considered in any ovine Johne's disease control or eradication program.

Fibre goat producers need to be aware that ovine Johne's disease may not be obvious in their animals. The disease is likely to be less frequently and less fully expressed than the disease in dairy

goats. A communication package needs to be developed to inform fibre goat producers of this fact and to encourage testing and participation in a market assurance program.

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