Scientific Article

Intra-uterine transmission of *Mycobacterium avium* subsp *paratuberculosis* in subclinically affected red deer (*Cervus elaphus*)

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Abstract

AIM: To determine the rate of transmission of *Mycobacterium avium* subsp *paratuberculosis* (*M. ptb*) from hind to fetus *in utero*, and the risk of transmission from dam to fawn via infected colostrum and milk in subclinically affected red deer hinds.

METHODS: Hinds were sourced from farms in Otago or Southland and selected for the study if they were positive to the immunoglobulin G1 (IgG1) modified enzyme-linked immunosorbent assay (ELISA) (Paralisa) and exhibited no clinical signs of Johne's disease. The hinds (n=35) were sent to a deer slaughter premises (DSP; n=31) or were killed on-farm (n=4). All post-mortem samples were collected from the fetus first and then from the dam, taking care to avoid cross contamination between samples. Fresh samples (n=185) were collected for culture, and tissue samples (n=72) were collected from 24 hinds and their fetuses for histopathological examination.

RESULTS: A total of 24/35 hinds selected were suitable for inclusion in the study. Eighteen of these pregnant hinds were culture-positive for *M. ptb*, and 14 of these had culture-positive fetuses, representing a transmission rate of 78% (95% confidence interval (CI) =0.58–0.98) from dam to fetus. Of the 16 mammary glands sampled, 11 (69%) were culture-positive for *M. ptb* while 12/15 (80%) mammary lymph nodes sampled were also culture-positive.

CONCLUSIONS: This study demonstrated a high rate of transmission of *M. ptb* from dam to fetus in red deer, and a potential risk of transmission to fawns suckling from mothers that are subclinically affected with Johne's disease.

KEY WORDS: ***Red deer, Cervus elaphus, Johne's disease, Mycobacterium paratuberculosis, intra-uterine transmission, mammary gland***

Introduction

Johne's disease, caused by *Mycobacterium avium* subsp *paratuberculosis* (*M. ptb*), has become a serious problem for the deer industry in New Zealand. Since the year 2000, there has been an increase in the number of microbiologically confirmed cases of Johne's disease in farmed deer in New Zealand, and the number of known-infected herds has risen from 299 to over 600 (de Lisle et al 2003, 2006). In sheep and cattle, most cases of Johne's disease are sporadic and occur in animals >3 years of age, whereas in deer, Johne's disease can occur as outbreaks in young deer 8–15 months of age and mortality rates as high as 20% have been reported (Mackintosh et al 2004). In addition, sporadic cases occur in adult deer but these only result in about 1–3% of animals being affected.

The economic impact of the disease occurs both on-farm and at slaughter. Impacts include production losses due to mortality, ill-thrift and reduced growth rates, poor reproductive performance, and interference with testing for bovine tuberculosis (Tb) (Mackintosh et al 2004). Johne's disease can result in false-positive reactions to the mid-cervical skin test for Tb in deer, which necessitates either further testing or the deer being sent to a DSP as a reactor. Granulomatous lesions caused by Johne's disease are treated at slaughter as suspect Tb lesions, resulting in the sale of detained carcasses to local markets instead of the premium export market.

Both 'ovine' and 'bovine' strains of Johne's disease can infect red deer (*Cervus elaphus*) in New Zealand, but the 'bovine' strain is more common (de Lisle et al 2006) and appears to be more pathogenic for deer than the ovine strain (O'Brien et al 2006; Mackintosh et al 2007). Clinically affected deer usually exhibit weight loss, ill-thrift, muscle wasting, and sometimes scouring. However, some infected deer do not exhibit any obvious clinical signs, but may still be significant shedders of *M. ptb* organisms.

Transmission of Johne's disease generally occurs via the faecal-oral route (Valentin-Weigand and Goethe 1999). This can occur from contaminated pastures or from milk or colostrum containing *M. ptb*. However, intra-uterine transmission in clinically affected animals has been demonstrated in cattle (Pearson and McClelland 1955; Lawrence 1956; McQueen and Russell 1979) and sheep (Lambeth et al 2004), and recently in deer (Deutz et al 2003; van Kooten et al 2006). In sheep and cattle, it has been shown that transmission to the fetus occurs in <10% of subclinically affected

AFO	Acid-fast organism
CI	Confidence interval
DSP	Deer slaughter premises
ELISA	Enzyme-linked immunosorbent assay
H&E	Haematoxylin and eosin
ICLN	Ileocaecal lymph node
ICV	Ileocaecal valve
IgG1	Immunoglobulin G1
JJ	Jejunum
JJLN	Jejunal lymph node
JPPA	Johne's protoplasmic antigen
JPPD	Johne's purified protein derivative
M. ptb	*Mycobacterium avium* subsp *paratuberculosis*
Tb	Bovine tuberculosis
ZN	Ziehl-Neelsen

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animals (Kopecky et al 1967; Sweeney et al 1992; Lambeth et al 2004), but to date there has been no research on the rate of transmission to the fetus in subclinically affected deer. This study was primarily undertaken to determine the rate of transmission from subclinically affected hinds to their fetuses. Previous studies have established that *M. ptb* can be isolated from the mammary glands, mammary lymph nodes, colostrum or milk of subclinically infected cattle (Sweeney et al 1992; Streeter et al 1995) but there are no similar data from deer. This study also examined the potential risk of transmission via infected milk or colostrum to the fawn.

Materials and methods

Selection of animals

The 35 animals used in this study were made available by their owners, and were selected using the following criteria: they had to be positive to the commercial IgG1 ELISA test (Paralisa; Disease Research Laboratory, University of Otago, Dunedin, NZ) for Johne's disease, not showing clinical signs of Johne's disease (no scouring, marked weight loss or muscle wasting), and to have been running with a stag during the rut and preferably confirmed as pregnant using ultrasound scanning. However, one animal from the 35 animals provided, which did not have a positive Paralisa test, was inadvertently added by the farmer.

The DeerQA Transport Programme (Deer Industry New Zealand) recommendations for the transport of pregnant hinds, and limited available killing space at a DSP, restricted the time-frame of the study to August and September 2005.

Sources of animals

The majority of hinds on Farm A were screened in early June using the Paralisa test, and a number of positive animals were found. Positive animals with high, medium and low Paralisa titres were selected in July. These were split into two groups; the first 'mid-pregnancy' group of 15 hinds was killed at a DSP on 04 August (Farm A1) and the second 'late pregnancy' group on 12 September (Farm A2), also at a DSP (Table 1). Half of the hinds from the second group of animals developed clinical Johne's disease over the winter, characterised by severe weight loss, muscle wasting and some scouring, reducing the number of subclinically affected animals to six individuals for the later group. An additional hind (Hind 13), which had not been Paralisa-tested previously, was inadvertently slaughtered with this second group.

Some deer on Farm B were positive to a whole-herd Tb skin test, and subsequent blood testing suggested that some of these were probably infected with *M. ptb*. Four of these hinds were killed on-farm as Tb reactors and their lymph nodes were examined for tuberculous lesions, as well as samples collected for this study.

Farm C had not experienced any clinical cases of Johne's disease, but the herd was screened with the Paralisa test in order to determine the infection status. Of the 600 hinds tested, 10 were positive to the test and were killed as part of this study, at a DSP, on 19 September (Table 1).

Serology

Animals were tested twice; first during the screening process on-farm and again immediately before slaughter. Blood samples were taken by jugular venepuncture, using a plain or heparinised vacutainer and 18-G needle, or at exsanguination at the DSP. Serum or plasma was tested using the IgG1 ELISA (Paralisa), using two antigens *viz* Johne's protoplasmic antigen (JPPA) and Johne's purified protein derivative (JPPD), using cut-off points of ≥40 and ≥60 optical density units, respectively. These were read in parallel, such that if either was positive the test was considered positive (Griffin et al 2005). The Paralisa test results were graded according to an arbitrary scale as high (JPPA >150 and JPPD >120), medium (JPPA 100–150 and JPPD 70–150), and low (JPPA 50–100 and JPPD 50–100), and similar numbers of deer in each category were chosen for the study.

Selection and collection of samples

At slaughter, at the DSP or on-farm, the uteri and intestines were collected and placed in separate sterile containers for later processing, as were mammary glands and lymph nodes, and hepatic lymph nodes. After slaughter, all samples were examined and sampled in a clean environment, to reduce the risk of cross contamination, and the fetuses were all examined before tissues from the hinds. The uterus was carefully opened and excess fluid drained out. The fetus was photographed, sexed and examined for any abnormalities, and its crown-to-rump length measured. To maintain consistency with previous studies (Deutz et al 2003; van Kooten et al 2006), pooled 1 x 1-cm samples of jejunum (JJ) and the entire ileocaecal valve (ICV), the entire jejunal lymph node (JJLN) chain, and a 1 x 1-cm tissue sample of each fetal lung were collected for culture. A 1 x 1-cm section of lung tissue was also collected and placed into 10% buffered formalin, for histopathological examination. The stainless-steel bench was cleaned with hot water, and the instruments sterilised between fetuses and, later, between the hinds.

The intestines from the hinds were sampled after all fetuses had been sampled and discarded. The JJLNs and the JJ were examined for gross signs of Johne's disease, and then fresh samples were collected from the JJLN; ileocaecal lymph node (ICLN); anterior, mid-, and posterior JJ; and the ICV, for culture. Samples from the anterior, mid- and posterior JJ with attached JJLNs, as well as the ICV with attached ICLNs, were collected and fixed in 10% buffered formalin, for histopathological examination.

Processing of samples

Culture

The sets of fresh samples were sent on ice by overnight courier and cultured the next day, using a modification of the BACTEC method described by Whittington et al (1999). Briefly, homogenised lymph node and fetal tissues were decontaminated with 0.75% cetylpyridinium chloride (Sigma-Aldrich, St Louis, USA) for 40 min, and intestinal samples were decontaminated overnight. They were then centrifuged at 3,500g for 20 min at 15°C, inoculated into supplemented BACTEC vials, and incubated at 37°C for a maximum of 60 days. Once a week, the vials were examined for the presence of bacterial growth. Vials with positive growth were examined for the presence of *M. ptb*, using the indices of slow growth, acid-fast staining and mycobactin dependence. One *M. ptb* isolate from each farm was sub-typed, using a highly specific polymerase chain reaction test, as described by de Lisle et al (2006).

Histopathology

Fixed samples of JJ, JJLNs, ICV, and ICLNs were processed routinely, and sections were stained with Ziehl-Neelsen (ZN) and haematoxylin and eosin (H&E). ZN-stained sections were examined for the presence of acid-fast organisms (AFOs), whereas H&E slides were examined to assess the severity of lesions charac-

teristic of Johne's disease, and were graded, with no knowledge of necropsy findings, using the scale presented in Table 2. This scale could not be used for grading fetal tissues, because neither AFOs nor granulomas were seen, and the following grading scale was used: Grade 0 = no inflammatory changes; Grade 1 = occasional neutrophils; Grade 2 = small numbers of neutrophils; Grade 3 = moderate numbers of neutrophils; and Grade 4 = marked numbers of neutrophils. The histopathology scores given in Table 1 for both the hind, and fetus, represent the worst affected tissue sample.

Statistical analysis

Where there were sufficient data, such as the proportion of positive hinds that had positive fetuses, these were analysed using a 95% CI that was calculated using the method developed by Clopper and Pearson (1934). Comparisons of proportions of animals in various sero-positive categories were made using a binomial-logit generalised linear model (McCullagh and Nelder 1989). Correlation coefficients were calculated between hind variables (time to become culture-positive and lesion severity score) and corresponding fetus variables. Analyses were conducted using GenStat for Windows Release 8.11 (VSN International Ltd, Oxford, UK).

Results

An unexpectedly high proportion (11/35; 31%) of hinds selected for this study were found to be not pregnant at slaughter, namely 3/15, 3/6, 2/4 and 3/10 hinds from Farms A1, A2, B and C, respectively (Table 1), compared with normal rates of <10% non-pregnant hinds expected by the farmers on these farms. Because of this, only 24/35 hinds selected were suitable for this study.

At slaughter, four of the hinds from Farm A had obvious gross lesions, while six others had suspicious signs of Johne's disease, including slight thickening of the posterior and/or mid-JJ and small white swellings in the mesenteries joining the JJ and JJLNs; there were no gross abnormalities seen in their fetuses. No gross lesions were seen in either the hinds or fetuses from Farms B and C.

Serology

Changes in the results of Paralisa tests carried out on plasma collected at slaughter compared with those of serum samples collected 6–10 weeks earlier are presented in Table 1. Three positives in the initial test were found to be negative at slaughter, and one (Hind 13) that had not been tested originally was negative in the Paralisa test at slaughter. However, all four of these hinds were subsequently found to be culture-positive.

Culture

Of the 24 hinds sampled for the study, 18 (75%) were culture-positive for *M. ptb*, including all the hinds from Farms A and B and one from Farm C (Table 3). The ICLN plus JJLN pool from the hinds returned 100% positive cultures for all 18 hinds (Table 3). Hepatic lymph nodes and JJ plus ICV returned 15/18 (83%) and 13/17 (76%) positive cultures, respectively. Mammary lymph nodes were not collected at slaughter from three of the hinds from Farm A, which left only 15 samples from culture-positive animals; of these, 12 (80%) were culture-positive while 11/16 (69%) of the mammary glands were positive.

Of the 18 culture-positive hinds, 14 (78%) had culture-positive fetuses (95% CI=0.58–0.98). All the culture-negative hinds had a culture-negative fetus. The culture success rate of tissues from the fetus was relatively high for the JJLNs plus ICLNs (77%) and JJ plus ICV (71%), compared with the lung (29%).

The time for the cultures to become positive is shown in Table 3. The JJLN plus ICLN samples from the hinds were the fastest to become positive and had an average time of 11 days, while the hepatic lymph nodes were next fastest with an average time of 15 days. The cultures from the fetuses averaged 28.4, 29.75 and 29.9 days for the JJ plus ICV, lung, and JJLN plus ICLN samples, respectively. The correlation coefficient between the time for cultures to become positive for the 14 hinds and their fetuses was not significant ($p>0.05$).

Of the 15 hinds that had positive cultures from their hepatic lymph nodes, 14 (93%) had culture-positive fetuses; there were no culture-positive fetuses from hinds that were culture-negative from their hepatic lymph nodes. The strains of *M. ptb* isolated on Farms A and B were identified as 'bovine', while the single isolate from Farm C was identified as the 'ovine' strain.

Histopathology

Histopathology results showed that seven hinds had severe Johne's disease lesions (Scores 10–12), six hinds had moderate pathology (Scores 5–6), three hinds had mild non-specific pathology (Scores 1–2), and no lesions or AFOs (Score 0) were observed in eight hinds (Table 1). None of the fetuses had any visible AFOs or any signs specific for Johne's disease. The majority had no inflammatory changes and 10 had mild inflammatory signs, but the lesion severity score of the hinds was not significantly correlated with that of the fetuses ($p>0.05$) and did not differ significantly with the infection status of the fetus ($p>0.05$).

The four culture-positive hinds that had culture-negative fetuses had histopathology lesion severity scores of 0, 1, 6 and 12, which was the same range as for culture-positive hinds and fetuses.

Discussion

This study demonstrated that there is a high risk of transmission of *M. ptb* to the fetus during pregnancy in subclinically affected hinds. Of the 24 hinds sampled in this study, 18 were infected with *M. ptb* and 14 of their fetuses were infected, giving a 78% (95% CI=0.58–0.98) rate of transmission. A similar study found a transmission rate of 88% in nine clinically affected hinds (van Kooten et al 2006). Both that study and our study demonstrated that transmission of *M. ptb* from dam to fetus could be relatively common in deer herds in New Zealand that are affected by Johne's disease, and could play an important role in the epidemiology of the disease.

Culture was the most reliable method for detecting *M. ptb* infection in deer in this study. The mesenteric lymph nodes (JJLNs plus ICLNs) were the best tissue for culture for both the hind and fetus, while the intestinal samples (JJ plus ICV) and lung samples had lower success rates. This may have been due to the number of organisms present in these tissues, site variation during sampling, or the longer decontamination process used for intestinal samples compared with lymph node samples before culturing. The isolation of *M. ptb* from the hepatic lymph node indicates that the infection had spread from the intestinal tract and draining lymph nodes and become systemic, and may have spread to the uterus and mammary gland.

Table 1. Animal identification (ID), dates of slaughter, farm of origin, post-mortem diagnosis and results of Paralisa tests and culture, and histopathological (Histo) severity score from hinds and fetuses that were affected by *Mycobacterium paratuberculosis*.

Hind ID	Date	Farm	Paralisa[a]	Paralisa[b]	Post-mortem diagnosis (hind)	Histo severity score (hind)[c]	Culture (hind)	Culture (fetus)
1	04 Aug	A1	High	Neg	Sus	2	Pos	Pos
2	04 Aug	A1	High	Pos	Sus	12	Pos	Pos
3	04 Aug	A1	Low	Pos	Lesion	5	Pos	Pos
4	04 Aug	A1	Med	Pos	Lesion	2	Pos	Pos
5	04 Aug	A1	Med	Pos	NVL	6	Pos	Neg
6	04 Aug	A1	High	Pos	NVL	6	Pos	Pos
7	04 Aug	A1	Low	Neg	NVL	5	Pos	Pos
8	04 Aug	A1	Low	Neg	Lesion	5	Pos	Pos
9	04 Aug	A1	High	Pos	Sus	12	Pos	Neg
10	04 Aug	A1	Med	Pos	NVL	12	Pos	Pos
11	04 Aug	A1	Med	Pos	Sus	10	Pos	Pos
12	04 Aug	A1	High	Pos	NVL	12	Pos	Pos
13	12 Sep	A2	ND[d]	Neg	Sus	0	Pos	Pos
14	12 Sep	A2	Med	Pos	Sus	11	Pos	Pos
15	12 Sep	A2	High	Pos	Lesion	5	Pos	Pos
16	14 Sep	B	High	Pos	NVL	10	Pos	Pos
17	14 Sep	B	Low	Pos	NVL	1	Pos	Neg
18	19 Sep	C	Low	Pos	NVL	0	Pos[e]	Neg
19	19 Sep	C	Low	Pos	NVL	0	Neg	Neg
20	19 Sep	C	Low	Pos	NVL	0	Neg	Neg
21	19 Sep	C	Low	Pos	NVL	0	Neg	Neg
22	19 Sep	C	Low	Pos	NVL	0	Neg	Neg
23	19 Sep	C	Low	Pos	NVL	0	Neg	Neg
24	19 Sep	C	Low	Pos	NVL	0	Neg	Neg

[a] Results classified as low, medium or high (positivity)
[b] Carried out on plasma collected at slaughter
[c] Scores are for the most severely affected tissue
[d] Animal inadvertently added into trial by farmer
[e] 'Ovine' strain typed
Neg = negative; Sus = signs suspicious (of Johne's disease); Pos = positive; Lesion = lesions found at slaughter; NVL = no visible lesions; ND = not done

Table 2. Overall lesion descriptions, scale of histopathological lesion severity, and absence or presence of acid-fast organisms (AFOs), in samples of jejunum, ileum, ileocaecal valve, jejunal lymph nodes and ileocaecal lymph nodes of red deer suspected of having subclinical Johne's disease.

Grade	Pathological lesions
0	No lesions or AFOs seen
1	Occasional Langhans giant cell seen or scattered macrophages in villi and no AFOs seen
2	Occasional granuloma within a small intestine or lymph node section only seen within one area of the intestine (e.g. posterior jejunum) and no AFOs seen
3	Occasional granuloma within small intestine or lymph nodes and no AFOs seen
4	Occasional granuloma within small intestine or lymph nodes plus AFOs seen
5	Occasional to scattered islands of granulomas within small intestine or lymph nodes and Grade-1 villi[a]
6	Scattered islands of granulomas within small intestine or lymph nodes and Grade-2 villi
7	Scattered islands of granulomas within small intestine or lymph nodes and Grade-3 villi
8	Scattered islands of granulomas within small intestine or lymph nodes, submucosal lesions and Grade-3 villi
9	Large areas of granulomatous lesions in intestine or lymph node, submucosal lesions and Grade-3 villi
10	Scattered areas of granulomatous lesions in intestine or lymph node, submucosal lesions, Grade-2 villi and mesenteric/pericapsular lymph node granulomas
11	Scattered areas of granulomatous lesions in intestine or lymph node, submucosal lesions, Grade-3 villi and mesenteric/pericapsular lymph node granulomas
12	Large areas of granulomatous lesions in intestine or lymph node, submucosal lesions, Grade-3 villi and mesenteric/pericapsular lymph node granulomas

[a] Grade-1 villi = mild blunting and fusion of villi; Grade-2 villi = moderate blunting and fusion of villi; Grade-3 villi = marked blunting and fusion of villi

Although *M. ptb* was isolated from fetuses in both this and the previous study by van Kooten et al (2006), the numbers of organisms appeared to be small in most cases, as indicated by a prolonged time to become positive in the BACTEC system. It has been shown by Reddacliff et al (2003) that the time to detect growth of *M. ptb* in BACTEC was inversely correlated with the number of organisms in the inoculum. However, the significance or consequences of intra-uterine infection in deer have not been determined, and it is not known if this will lead to the early development of immune tolerance and a carrier state, the rapid development of clinical disease in the fawn, or some other outcome.

Mycobacterium paratuberculosis was isolated from only one of the seven pregnant hinds from Farm C, and was typed as an 'ovine' strain. There was no pathology detected in this hind and the fetus was culture-negative. Previous studies have shown that the ovine strain is much less pathogenic for red deer than the bovine strain (O'Brien et al 2006; Mackintosh et al 2007). These hinds came from a herd of 600 animals that were screened with the Paralisa test, resulting in 10 positive animals (1.7% of the herd), which is close to the expected number of false-positives for the Paralisa test (specificity of ~99%) (Griffin et al 2005).

Hinds 1, 7, and 8 were Paralisa test-positive in the original on-farm screening test but subsequently had reverted to a negative status at slaughter. The sensitivity of the Paralisa test has been estimated at ~90%, using a dataset of 102 infected animals from a number of high-prevalence deer herds (Griffin et al 2005). However, it has also been shown (Mackintosh et al 2007) that the Paralisa test status of experimentally infected deer can fluctuate between positive and negative over time, and this appears to be the case for the animals in this present study. This may also be the case for Hind 13, which was also negative at slaughter but had a disseminated infection.

There were insufficient data to analyse possible associations between the results of serology, culture or histopathology. There were also insufficient data to determine if there were any correlations between infection rates in fetuses and the severity of histopathology in the hinds, or differences between hinds slaughtered in August compared with September.

Mammary gland tissue and mammary lymph nodes were examined during this study, but milk and colostrum were not collected because the hinds were killed before lactogenesis took place. However, other studies with dairy cows suggest that milk and colostrum can become contaminated when the associated lymph nodes are infected (Sweeney et al 1992). Of the 18 (75%) culture-positive hinds, 15 (83%) had their mammary lymph nodes examined and 12 (80%) of these were positive, while mammary tissues from 16 hinds yielded 11 (69%) positive cultures. These results suggest that if a fawn born to a subclinically affected hind is not infected *in utero*, there is a relatively high risk that it will be exposed to infection via colostrum and milk from birth, when it is

Table 3. Culture results for pooled jejunal lymph nodes plus ileocaecal lymph nodes (JJLN+ICLN), pooled jejunum plus ileocaecal valve (JJ+ICV), hepatic (Hep) lymph nodes (LN), mammary LN and mammary gland (GL) samples from the hinds, and pooled JJLN+ICLN, pooled JJ+ICV, and lung samples from the fetuses. Weeks to culture-positive for JJLN+ICLN, pooled JJ+ICV, Hep LN, mammary LN and mammary GL samples from the hinds, and pooled JJLN + ICLN, pooled JJ + ICV, and lung samples from the fetuses are included in brackets.

	Hind					Fetus		
Hind	JJLN+ICLN	JJ+ICV	Hep LN	Mammary LN	Mammary GL	JJLN+ICLN	JJ+ICV	Lung
1	Pos (2)	Pos (2)	Pos (4)	Pos (5)	Pos (6)	Pos (4)	Pos (5)	Neg
2	Pos (1)	Pos (1)	Pos (1)	Pos (3)	Pos (4)	Pos (4)	Pos (5)	Neg
3	Pos (1)	Pos (1)	Pos (3)	[a]	[a]	Neg	Pos (5)	Neg
4	Pos (3)	Pos (3)	Pos (5)	Pos (4)	Pos (4)	Pos (5)	Neg	Pos (5)
5	Pos (1)	Pos (2)	Neg	Pos (5)	Pos (5)	Neg	Neg	Neg
6	Pos (1)	Neg	Pos (1)	Pos (5)	Pos (4)	Pos (5)	Neg	Neg
7	Pos (2)	Neg	Pos (2)	Pos (2)	Pos (4)	Neg	Pos (4)	Neg
8	Pos (1)	[a]	Pos (1)	Pos (4)	Pos (3)	Pos (5)	Pos (3)	Neg
9	Pos (1)	Pos (1)	Pos (2)	Pos (4)	Pos (4)	[a]	Neg	Neg
10	Pos (1)	Pos (1)	Pos (1)	Pos (4)	Pos (2)	Neg	Pos (4)	Pos (5)
11	Pos (1)	Pos (3)	Pos (2)	Pos (2)	Pos (5)	Pos (5)	Pos (5)	Neg
12	Pos (1)	Pos (1)	Pos (1)	[a]	Pos (4)	Neg	Pos (5)	Pos (5)
13	Pos (2)	Pos (4)	Pos (2)	Pos (1)	Neg	Pos (2)	Pos (3)	Pos (2)
14	Pos (1)	Neg	Pos (1)	[a]	[a]	Pos (1)	Pos (1)	Neg
15	Pos (1)	Pos (2)	Pos (2)	Pos (4)	Neg	Pos (6)	Neg	Neg
16	Pos (1)	Pos (1)	Pos (2)	Neg	Neg	Pos (5)	Neg	Neg
17	Pos (4)	Pos (5)	Neg	Neg	Neg	Neg	Neg	Neg
18	Pos (3)[b]	Neg	Neg	Neg	Neg	Neg	Neg	Neg
19	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
20	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
21	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
22	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
23	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
24	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

[a] No result due to contamination or no sample cultured
[b] 'Ovine' strain typed
Pos = positive; Neg = negative

likely to be highly susceptible. There is also a possibility of fawns becoming infected through mis-mothering or cross-suckling from infected hinds.

During this study, it was also noted that the subclinically affected hinds had a lower pregnancy rate than unaffected animals from the farms. Only 24/35 (69%) animals were pregnant, which is lower than the usual pregnancy rates of 85–90% of the farms involved in this study. It is possible that subclinical infection with Johne's disease may result in a lower fawning percentage.

In conclusion, this study has shown that there was a high rate of transmission of *M. ptb* from subclinically affected hinds to their fetuses. The study also demonstrated that there is a possible risk of transmission from infected colostrum and milk. Subclinically affected hinds should therefore be culled as soon as they are identified as infected, to reduce the prevalence of infection in the next generation of fawns.

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