Intrauterine and transmammary transmission of *Mycobacterium avium* subsp *paratuberculosis* in sheep

C LAMBETH[a], LA REDDACLIFF[b], P WINDSOR[a], KA ABBOTT[c], H McGREGOR[a] and RJ WHITTINGTON[a]

Objective To investigate intrauterine infection of foetuses with *Mycobacterium avium* subsp *paratuberculosis* and the presence of infection in mammary secretions of sheep.

Design A study of 142 late-pregnant ewes and their foetuses from two heavily infected flocks.

Procedure Infection of ewes was determined at necropsy by histopathology and culture of tissues and mammary secretions. Antemortem tests (clinical assessment, faecal culture and serology) were also applied. Foetuses from 59 infected ewes and 47 apparently uninfected ewes were examined by culture and histopathology.

Results Five of five ewes with clinical ovine Johne's disease had infected foetuses. Only one of 54 subclinically affected ewes, and none of 47 uninfected ewes had an infected foetus. *M a paratuberculosis* was cultured from mammary secretions or mammary glands of only two of 76 ewes, both of which were clinical cases and had infected foetuses.

Conclusion Although intrauterine or transmammary transmission of *Mycobacterium avium* subsp *paratuberculosis* may occur frequently in clinically affected sheep, these are less common in subclinically infected ewes. Therefore these modes of transmission are unlikely to compromise existing control programs for ovine Johne's disease on most farms, especially if programs include the immediate culling of clinically affected sheep.

Aust Vet J 2004;82:504-508

AGID	Agar gel immunodiffusion
HPC	Hexadecylpyridinium chloride
ICV	Ileocaecal valve
M a paratuberculosis	*Mycobacterium avium* subsp *paratuberculosis*
MLN	mesenteric lymph node(s)
OJD	Ovine Johne's disease
SLN	Supramammary lymph node
TI	Terminal ileum

Control of OJD, as distinct from eradication, depends on accurate information on the mode of transmission of infection between sheep. This is especially important to enable the development of strategies to reduce disease on individual properties, for trading sheep from infected properties and for infected studs seeking to eliminate OJD while preserving their genetic base.

[a]Faculty of Veterinary Science, University of Sydney, Private Bag 3, Camden, New South Wales 2570
[b]NSW Agriculture, EMAI, PMB 8 Camden, New South Wales 2570 (author for correspondence)
[c]Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts. AL9 7TA, UK

Faecal excretion is the main source of environmental contamination with *M a paratuberculosis*, and is probably the main means of transfer of infection between animals. Exposure to infected faeces can occur by ingestion of contaminated pasture, soil, or water, or in suckling animals, from faeces on the teats.[1] Clinically affected sheep can excrete enormous numbers of organisms in their faeces,[2] and faecal contamination from even a single clinical case may be sufficient to infect many susceptible animals. Studies in both cattle and sheep indicate that young animals are the most susceptible, whereas adults require higher doses and longer incubation periods to show signs of disease.[3-5]

Current strategies to reduce transmission are based on the above knowledge, and are designed to avoid exposure of young lambs to potentially infected adults. Such practices include artificial rearing of lambs, rearing young sheep away from adults or pasture grazed by adults, and rearing of rams in sheds. However, these methods all rely on the assumption that lambs are unlikely to become infected by intrauterine or transmammary transmission, and to date there is little information specific to sheep on which to base this assumption.

Reports suggest that 26 to 35% of foetuses from clinically affected cows are infected with *M a paratuberculosis*,[6 7] while about 10% of foetuses from subclinically infected cows are infected.[8] There are few references to possible foetal infection in sheep. In one study, acid-fast bacilli were cultured from the hepatic lymph nodes of a foetus from a sub-clinically infected ewe, and *M a paratuberculosis* was identified in the uterine mucosa of 4 ewes which were positive in the complement fixation test.[9] The detection of antibodies to *M a paratuberculosis* using double immunodiffusion and counterimmunoelectrophoresis tests in 3% of lambs before suckling from seropositive ewes is also suggestive of intrauterine exposure.[10] Most sheep strains of *M a paratuberculosis* have until recently been difficult to culture in vitro,[11] which might lead to under-reporting of foetal infection in this species, given that foetal infection is usually without lesions.[12]

There are also a number of reports of possible transmammary infection in cattle. Several investigations have shown that subclinically infected cows can excrete *M a paratuberculosis* in their milk. One study found that 22% excreted the organism in their colostrum and 3% in milk.[13] Another found 12% of subclinically infected cows were shedding *M a paratuberculosis* in milk and that prevalence of excretion in milk correlated with the amount of shedding in faeces.[14] Clinically affected cows are even more likely to excrete the organism in milk, with one study showing a prevalence of 45%.[15] There are no references concerning the culture of *M a paratuberculosis* from the milk of sheep. However, the situation may be similar to that in cattle, as shown by a recent study which detected *M a paratuberculosis* DNA by PCR in 88% of milk samples from sheep that were positive in the gamma-interferon test.[16] The numbers of organisms in infected milk may be very low compared to the numbers excreted in faeces. A single

study in subclinically infected cattle found only 2 to 8 colony-forming-units per 50 mL.[14] More *M a paratuberculosis* organisms are present in colostrum, probably due to its containing large numbers of macrophages.[17] Colostrum is available to the neonate when it is most susceptible to infection, and specific antibodies in colostrum of animals with antibodies *M a paratuberculosis*, have been shown to increase the uptake of *M a paratuberculosis* by intestinal M cells.[18] Thus, neonatal infection may be more likely to occur from the ingestion of infected colostrum rather than milk, but the significance of mammary excretion of *M a paratuberculosis* in the epidemiology of ovine Johne's disease is unclear.

The primary aim of this study was to determine the probability of intrauterine or transmammary infection from subclinically infected Merino sheep to their lambs. This is important because it is a potential source of breakdown in OJD control programs. For comparison, a small number of clinically affected pregnant ewes were included in the study, although clinically affected sheep would usually be culled in a control program.

Materials and methods

Animals

The main study was conducted on Farm A, located near Goulburn on the Southern tablelands of New South Wales. This farm had a high prevalence of *M a paratuberculosis* infection. Annual mortalities of up to 20% from OJD had been recorded, and 8 to 16% of clinically normal 2-3-year-old sheep were positive in the AGID test. In August 2000, 159 3-year-old ewes were randomly selected for this study, and were grazed as a single mob. In August 2001, blood and faecal samples were collected from the 145 surviving ewes, now 4-years-old, and then necropsy examinations were performed during four farm visits over a two week period. Nine non-pregnant sheep were excluded, leaving 136 ewes in the study. The age of foetuses at the time of necropsy ranged from 95 to 149 days, based on crown-rump length. On a second farm (Farm B), six late-pregnant ewes with clinical signs suggestive of OJD were necropsied.

Agar gel immunodiffusion test (Farm A animals only)

Blood was collected into plain vacutainers, allowed to clot and retract at room temperature, then stored at 4°C. Serum was removed within 48 hours for testing using the AGID test.[19]

Necropsy samples

Duplicate samples of tissues were collected into sterile 5 mL containers for culture of *M a paratuberculosis,* and into 10% neutral buffered formalin for histopathology. The following tissues were collected from each ewe: ICV, TI (a pool of two samples taken at intervals of approximately 10 cm proximal to the ICV), MLN (a pool of three samples comprising an ileocaecal node, the caudal jejunal node and another more proximal node) and SLN. Mammary secretion was collected into a 10 mL sterile centrifuge tube for culture only. The following tissues were collected from each foetus: cotyledon, ICV, spleen and pools of TI and MLN as above. The ICV and TI samples from each ewe or foetus were pooled for a single ICV/TI culture. In addition, uterus, uterine lymph node and mammary gland from ewes, and liver and umbilicus from foetuses, were collected from the Farm B animals.

Necropsy procedure

Ewes were euthanased with intravenous injection of pentobarbitone and placed on their right side. Particular care was taken to avoid cross-contamination between samples. Separate sterile instruments were used for each foetus and each ewe, and ewe and foetal samples were collected by different operators. Chopping boards were scrubbed clean, then boiled in a steriliser for at least 10 minutes between each animal. New gloves were used for each animal. Samples from ewes were collected in the specific order below to avoid cross-contamination of mammary secretion from intestinal contents or MLN. The mammary gland and inguinal region were thoroughly scrubbed with medicated soap and water, to remove possible faecal contamination.

Samples from foetus — The flank of the ewe was incised, the uterus exteriorised, then incised along the curvature of the spine of the foetus. The foetus was exteriorised onto a chopping board and euthanased if necessary with an intracardiac injection of pentobarbitone. Following collection of a foetal cotyledon, the umbilical cord was severed and the foetus removed to a separate table before the remaining samples were taken. Initially, samples were collected from all foetuses, but subsequently, due to a high rate of twinning, only one foetus per ewe was sampled, except when the ewe had gross lesions indicative of OJD.

Samples from ewes — After removal of the foetus, an incision was made 2 to 3 cm above the base of the teat, and up to 10 mL of mammary secretion was collected. The secretion frequently had a thick honey-like consistency and was difficult to collect using a syringe. In these cases, a sterile needle cap was used to scoop out as much secretion as possible. Samples were often less than 1 mL in volume, with no secretion obtained from some ewes. The SLN was then collected. Finally, the abdominal cavity was inspected for gross lesions of OJD (thickening of TI/ICV, enlargement of MLN, cording of lymph vessels), and MLN then intestinal samples were collected.

Processing of mammary secretion and samples collected from foetuses

Tissues collected from foetuses were stored, then samples from selected foetuses were processed for culture and histopathological examination after the results of culture and histopathology from ewe samples were known. From Farm A, tissues were processed from all foetuses collected from infected ewes, and from foetuses from 47 apparently uninfected ewes. Tissues from all foetuses from the six Farm B ewes were also processed. Similarly, mammary secretions, were stored, then cultured from all infected ewes that yielded secretion, and from 32 uninfected ewes.

Histopathology

Fixed tissues were processed routinely for histopathology, then stained with hematoxylin and eosin and the Ziehl-Neelsen method. The lesions from ewes were graded using an adaptation of the classification of Perez,[20] with an additional category (2N) to include sheep with lesions in the MLN only.

Culture for M a paratuberculosis

Samples were initially held at 4°C, then frozen at -80°C within 10 hours, and stored for up to 6 months.

Preparation of tissues — These were prepared as previously described.[11] Briefly, each sample (2 to 5 g) was trimmed of excess fat, homogenised in 2 mL of sterile normal saline, then mixed with 25 mL of 0.75% HPC (Sigma Chemical Co., St Louis, MO) in a 35 mL polystyrene tube. This was left undisturbed at room temperature for 72 hours. A 100mL aliquot was removed from near the bottom of the tube by aspiration using a 25 gauge needle on a tuberculin syringe, and inoculated into a BACTEC vial. A modification of the tissue protocol, using a centrifugation step which provides greater sensitivity,[21] was used for tissues from

foetuses of 30 of the 47 uninfected ewes. For each of these foetuses, only two BACTEC cultures were done – cotyledon, and a pooled culture of TI/ICV, MLN and spleen.

Preparation of faeces — A double incubation method was used.[11] Briefly, 2 to 5 g of faeces was mixed with 10 to 12 mL of sterile normal saline in a 15 mL polypropylene tube. After mixing, the tube was allowed to stand for 30 min at room temperature. Five mL of fluid from near the surface was transferred to a 35 mL polystyrene tube containing 25 mL of 0.9% HPC in half-strength brain heart infusion broth (Oxoid, Basingstoke, England) and allowed to stand at 37°C for 24 h. The tube was then centrifuged at 900 *g* for 30 min. The pellet was resuspended in 1 mL of sterile water with vancomycin (100 µg/mL), nalidixic acid (100 µg/mL) and amphotericin B (50 µg/mL) and incubated for 72 h at 37°C. Sediment was then resuspended by vigorous agitation, and 100 mL was inoculated into a BACTEC vial.

Preparation of mammary secretion – Thawed samples were transferred to a new 10 mL sterile centrifuge tube and the volume made up to 10 mL with sterile phosphate buffered saline. Samples were mixed on a tube rotor at 37°C for 2 to 3 hrs, then centrifuged at 2500 *g* for 15 min. The supernatant was discarded and 5 mL of 0.75% HPC was added to the pellet. Pellets were resuspended by mixing on a tube rotor at 37°C for 1 to 2 hrs, then left undisturbed at room temperature for 72 hrs. 100mL was removed from near the bottom of the tube by aspiration using a 25 gauge needle on a tuberculin syringe, and inoculated into a BACTEC vial.

BACTEC culture — BACTEC vials were incubated at 37°C for up to 20 weeks. The modified BACTEC 12B radiometric medium consisted of 4 mL enriched Middlebrook 7H9 medium (BACTEC 12B; Becton Dickinson, Sparks, Md) with 200 µL PANTA PLUS (Becton Dickinson), 1 mL egg yolk, 5 µg of mycobactin J (Allied Monitor Inc, Fayette, MO) and 0.7 mL of water.[11] Growth indices were measured weekly with a BACTEC 460 machine (Johnston Laboratories, Towson, MD), and the presence of *M a* subsp *paratuberculosis* in growth index positive vials was confirmed using PCR for IS*900* and restriction endonuclease analysis.[11,22]

Classification of infected animals

The following criteria were used to classify sheep with regard to OJD:

Infected sheep — Any animal which had histopathological lesions consistent with OJD and/or had a positive culture (from tissues and/or faeces and/or milk) was classified as infected.

Clinical OJD case — Infected sheep with clinical signs indicative of OJD (emaciation, with or without diarrhoea) were classified as clinical cases only if their pathology was widespread and severe (Perez classifications 3b (severe, diffuse multibacillary) or 3c (severe, diffuse paucibacillary)).[20]

Subclinical OJD case — Infected sheep without clinical signs of OJD were classified as subclinical OJD cases. Infected sheep with clinical signs, but with lesions not sufficiently extensive to have caused the clinical signs, were also classified as subclinical cases.

Uninfected sheep — These animals were negative by histopathology and culture for OJD. While infection was not detected in these sheep, it is possible that some were infected in tissues not sampled, or infection in the sampled tissues was below the limits of detection.

Statistical analysis

The 95% binomial confidence intervals for the mean number of infected foetuses from ewes in the three categories of infection were calculated using Statmate (GraphPad Software, San Diego, CA 92121, USA).

Results

Ewes

Summary findings from the ewes is given in Table 1. Overall, 42% of ewes (59 of 142) were confirmed to be infected. Five ewes were classified as clinical OJD cases and 54 ewes were subclinical cases.

Culture results — M a paratuberculosis was cultured from at least one tissue from 35% of ewes (46 of 136 ewes from Farm A and 4 of 6 from Farm B). MLN cultures were positive in 37 ewes and ICV/TI in 33. SLN cultures were positive in three ewes, and these ewes had no histopathological lesions of OJD. In another ewe with no lesions, *M a paratuberculosis* was isolated only from the uterus. Mammary secretion and/or mammary gland was cultured from 76 ewes (3 clinical OJD cases, 41 subclinical cases and 32 uninfected). Only two positive cultures were obtained, both from the mammary tissue of clinical cases.

Histopathology — Lesions of OJD were present in 34.5% of ewes (45 of 136 from Farm A and 4 of 6 from Farm B). All but 9 of the histologically positive ewes were also culture-positive. These nine ewes had focal lesions only in the Peyer's patches of the intestine or in MLN (categories 1 or 2N).

Antemortem examination — M a paratuberculosis was cultured from the faeces of only 7% of ewes (9 of 136 from Farm A, and 1 of 4 from Farm B), and in each of these ewes, tissues were also culture-positive. Six of 136 sheep were positive in the AGID, with five of these confirmed as infected (four subclinical and one clinical). Twelve sheep had clinical signs consistent with OJD, but only eight of these were confirmed infected (five classified as clinical cases and three subclinical). Overall, antemortem examination detected only 30% of the subclinically infected ewes (16 of 54).

Foetuses

Samples from 119 foetuses out of 106 ewes were examined. These included six foetuses from five clinical cases, 63 foetuses from 54 subclinically infected ewes and 50 foetuses from 47 uninfected ewes.

Culture results — M a paratuberculosis was isolated from six foetuses. Foetal cotyledon was positive in all six cases, and was the only culture-positive tissue in four of these. In two foetuses, *M a paratuberculosis* was also isolated from other tissues (spleen, liver and umbilicus).

Histopathology — There were no significant lesions consistent with OJD in any foetus.

Table 1. Numbers of ewes from two farms classified as clinical cases, subclinical cases or uninfected with regard to OJD.

	Confirmed clinical cases	Subclinically infected	Not detectably infected	Total ewes
Farm A	2	52	82[a]	136
Farm B	3	2	1	6
Total	5	54	83	142

[a]Foetuses from only 47 of these ewes were examined.

Correlation of infection in ewes and foetuses

Table 2 shows the relationship between the OJD classification of ewes and the findings from their foetuses, and Table 3 shows the test results for all ewes which had infected foetuses. All five ewes in this study with confirmed clinical OJD had an infected foetus. In one ewe, only one of twin foetuses was infected, so that in total 83% of foetuses from the clinical OJD cases were infected. From the subclinically infected ewes, only 1.6% of foetuses were infected. *M a paratuberculosis* was not isolated from any of the foetuses from uninfected ewes.

Discussion

This study of vertical transmission of *M a paratuberculosis* was conducted in two flocks with a high prevalence of OJD, and all six ewes selected from Farm B had clinical signs indicative of OJD. Even so, only 5% of foetuses (6 of 119) were demonstrated to be infected, and all but one were from ewes classified as clinical cases of OJD. In four infected foetuses, cotyledon was the only tissue that was positive on culture, and this would almost certainly have contained some maternal tissue. Thus only two foetuses had unequivocal infection in foetal tissues, and both were from confirmed clinical cases.

Five of the 6 ewes with infected foetuses had severe diffuse histopathological lesions, typical of advanced OJD. Two of these were from Farm A. Both had clinical signs consistent with OJD, *M a paratuberculosis* was cultured from faeces, and one was positive in the AGID test. Both would have been readily detected by routine antemortem screening for OJD. The other 3 ewes with severe lesions were from Farm B, and all had clinical signs of OJD. Faecal culture results were available for only one (positive). Although the final ewe had clinical signs indicative of OJD, *M a paratuberculosis* was cultured only from the uterus, and there were no histopathological lesions of OJD, so it was classified as a subclinical case. No AGID result was available, and except for the clinical signs, the cause of which was not determined, it may not have been detected by routine antemortem tests for OJD.

M a paratuberculosis was not isolated from any of the samples of mammary secretion, but it was cultured from the mammary glands of two clinical cases from Farm B. A further three ewes were culture positive in the SLN with no other findings indicative of OJD, but the significance of this is unclear. The mammary secretion samples in this study were not ideal. Due to the range of gestational ages, samples varied from gelatinous secretion through to colostrum. The amount that could be collected ranged from less than 1 mL up to 10 mL, and the consistency of the samples made them difficult to collect. The culture method used for these samples probably had a similar analytical sensitivity to that routinely used for culture of tissues, about 10^2 – 10^3 organisms.[21] In similar studies on cattle, 50 mL samples were used and few organisms were isolated (2-8 colony-forming-units per 50 mL).[14] In the current study, the combination of small sample volume and culture sensitivity means that such low numbers of organisms would not have been detected. However, if infective dose of *M a paratuberculosis* in milk for sheep is similar to that demonstrated for experimental oral infection (> 10^4 organisms),[23] then infection via the milk is unlikely to be of practical significance in most flocks. Also, of the only two ewes in this study with positive cultures from milk or mammary tissue, foetuses were shown to be already infected. However, further studies, using samples from lactating ewes, are needed to more reliably determine the amount of transmammary transmission in sheep.

In a herd of dairy cattle, the use of infected pooled colostrum in calves could potentially disseminate infection rapidly, and pasteurisation does not completely remove *M a paratuberculosis* from colostrum.[17] However, in beef or sheep enterprises, where neonates usually remain with their dams, there is more risk of oral exposure to infective faeces, and whether or not infection occurs via the milk may be less important. In special cases, such as when small numbers of genetically valuable lambs were to be 'salvaged', colostrum should be obtained from tested uninfected ewes to reduce the risk of transmammary infection. Whilst possible congenital infection could never be completely avoided, the

Table 2. Comparison of foetal infection with the infection status of their dam.

Infection status of ewe	Number of ewes	Number of foetuses	Number of infected foetuses	% ewes with infected foetus	% infected foetuses (95% CI)
Clinical OJD	5	6	5	100	83 (36,100)
Subclinical	54	63	1	1.9	1.6 (0,9)
Uninfected	47	50	0	0	0 (0,7)
Subclinical or uninfected	101	113	1	1	0.9 (0,5)
Total	106	119	6	5.7	5.0 (2,11)

Table 3. Results of antemortem and postmortem tests for OJD from 6 ewes that had infected foetuses.

Farm	OJD category	Clinical signs	Histological findings[a]	Culture from tissues					Faecal culture	AGID test
				ICV/TI	MLN	SLN	other	Mammary gland or secretion		
A	Clinical	+	+ (3c)	+	+	-	ns	ns	+	-
A	Clinical	+	+ (3b)	+	+	-	ns	ns	+	+
B[b]	Clinical	+	+ (3c)	+	+	ns	+ (uterus)	+	ns	ns
B[b]	Clinical	+	+ (3b)	+	+	ns	+ (uterus)	-	ns	ns
B	Clinical	+	+ (3b)	+	+	ns	+ (uterine LN)	+	+	ns
B	Subclinical	+	-	-	-	ns	+ (uterus)	-	-	ns

[a]Lesion classification according to Perez[20] in parentheses.
[b]Foetuses from these ewes were culture-positive in tissues other than cotyledon.
+ = Positive
- = Negative
ns = No sample or results not available.

results from our study indicate that simply excluding ewes in poor body condition will greatly reduce the risk of foetal infection. Antemortem testing may further reduce that risk, although current tests will fail to detect a large proportion of subclinically infected sheep.

In both the current and reported studies of foetal *M a paratuberculosis* infection, there is no indication whether any of the infected foetuses may remain subclinical carriers or later progress to become clinical cases. The ovine foetus has been reported to be unable to mount an immune response to certain antigens, including BCG,[24] and this immaturity of the immune system may extend to *M a paratuberculosis*. As the immune system matures after birth it is possible that infection may be eliminated from some infected foetuses, or conversely, that some degree of tolerance may occur, increasing the likelihood of development of a carrier state. This has not been studied, but an observation made in 1935 that a calf born to a cow with clinical Johne's disease was skin test positive at one month of age and later developed Johne's disease, despite extreme precautions taken during the birth of the calf, suggests that at least some infected foetuses may later develop Johne's disease.[25]

The findings from our study reinforce current recommendations for the control of OJD in infected flocks. In flocks with a high prevalence of infection, it is advisable to immediately remove any clinically affected ewes from the flock, and not retain their progeny if these can be identified. This will substantially reduce the risk of the birth of an infected lamb and simultaneously reduce the amount of contamination of pastures with faeces containing *M a paratuberculosis*. The finding of a high amount of foetal infection in ewes with clinical OJD was not surprising, considering the published studies in cattle. The low amount of foetal infection in subclinically affected ewes and its absence in apparently uninfected ewes is more important. Considering these two groups together, only 0.9% (95% CI, 0 - 5) of foetuses were infected. Remembering that these were from flocks with a high prevalence of OJD, our findings provide some assurance that congenital infection is unlikely to be a significant barrier to existing control or stud recovery programs.

Acknowledgments

This work was funded by Meat and Livestock Australia, New South Wales Agriculture and Sydney University. Thanks are also due to Terry and Cecily Hayes from "Hillwood" for on-farm assistance, to the owners of Farm B, and to Shayne Fell, Vanessa Saunders and Anna Waldron for skilled technical assistance.

References

1. Whittington RJ, Sergeant ES. Progress towards understanding the spread, detection and control of *Mycobacterium avium* subsp *paratuberculosis* in animal populations. *Aust Vet J* 2001;79:267-278.
2. Whittington RJ, Reddacliff LA, Marsh I, McAllister S, Saunders V. Temporal patterns and quantification of excretion of *Mycobacterium avium* subsp *paratuberculosis* in sheep with Johne's disease. *Aust Vet J* 2000;78:34-37.
3. Larsen AB, Merkal RS, Cutlip RC. Age of cattle as related to resistance to infection with *Mycobacterium paratuberculosis*. *Am J Vet Res* 1975;36:255-257.
4. Whitlock RH, Buergelt C. Preclinical and clinical manifestations of paratuberculosis (including pathology). *Vet Clin North Am Food Anim Pract* 1996;12:345-356.
5. McEwen AD. Investigations on Johne's disease in sheep. *J Comp Pathol* 1939;52:69-87.
6. Doyle TM. Foetal infection in Johne's disease. *Vet Rec* 1958;70:328.
7. Seitz SE, Heider LE, Heuston WD et al. Bovine fetal infection with *Mycobacterium paratuberculosis*. *J Am Vet Med Assoc* 1989;194:1423-1426.
8. Sweeney RW, Whitlock RH, Rosenberger AE. *Mycobacterium paratuberculosis* isolated from fetuses of infected cows not manifesting signs of the disease. *Am J Vet Res* 1992;53:477-480.
9. Tamarin R, Landau M. Congenital and uterine infection with *Mycobacterium johnei* in sheep. *Refuah Veterinarith* 1961;18:43-44.
10. Muhammed SI, Eliasson EC. The prevalence of antibodies to *Mycobacterium johnei* in colostrum deprived lambs. *Vet Rec* 1979;105:11-12.
11. Whittington RJ, Marsh I, Turner MJ et al. 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* 1998;36:701-707.
12. Clarke CJ. The pathology and pathogenesis of paratuberculosis in ruminants and other species. *J Comp Pathol* 1997;116:217-261.
13. Streeter RN, Hoffsis GF, Bech-Nielsen S, Shulaw WP, Rings DM. Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. *Am J Vet Res* 1995;56:1322-1324.
14. Sweeney RW, Whitlock RH, Rosenberger AE. *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows. *J Clin Microbiol* 1992;30:166-171.
15. Giese SB, Ahrens P. Detection of *Mycobacterium avium* subsp *paratuberculosis* in milk from clinically affected cows by PCR and culture. *Vet Microbiol* 2000;77:291-297.
16. Grant IR, Pope CM, O'Riordan LM, Ball HJ, Rowe MT. Improved detection of *Mycobacterium avium* subsp *paratuberculosis* In milk by immunomagnetic PCR. *Vet Microbiol* 2000;77:369-378.
17. Meylan M, Rings DM, Shulaw WP et al. Survival of *Mycobacterium paratuberculosis* and preservation of immunoglobulin G in bovine colostrum under experimental conditions simulating pasteurization. *Am J Vet Res* 1996;57:1580-1585.
18. Momotani E, Whipple DL, Thiermann AB, Cheville NF. Role of M cells and macrophages in the entrance of *Mycobacterium paratuberculosis* into domes of ileal Peyer's patches in calves. *Vet Pathol* 1988;25:131-137.
19. Cousins DV, Condron RJ, Eamens GJ, Whittington RJ, De Lisle GW. Australian and New Zealand Standard Diagnostic Procedures. Paratuberculosis (Johne's Disease). http://www.scahls.com/jd.htm.2002. Retrieved 25 May 2004.
20. Perez V, Garcia Marin JF, Badiola JJ. Description and classification of different types of lesion associated with natural paratuberculosis infection in sheep. *J Comp Pathol* 1996;114:107-122.
21. Reddacliff LA, Vadali A, Whittington RJ. The effect of decontamination protocols on the numbers of sheep strain *Mycobacterium avium* subsp *paratuberculosis* isolated from tissues and faeces. *Vet Microbiol* 2003;95:271-282.
22. Cousins DV, Whittington R, Marsh I et al. Mycobacteria distinct from *Mycobacterium avium* subsp *paratuberculosis* isolated from the faeces of ruminants possess IS*900*-like sequences detectable by IS*900* polymerase chain reaction: implications for diagnosis. *Mol Cell Probes* 1999;13:431-442.
23. Reddacliff LA, Whittington RJ. Experimental infection of weaner sheep with S strain *Mycobacterium avium* subsp *paratuberculosis* . *Vet Microbiol* 2003;96:247-258.
24. Sheep immunology and goat peculiarities. in: Pastoret P, Griebel P, Bazin H, Govaerts A, Editors. Handbook of vertebrate immunology. San Diego, London: Academic Press, 1998:517.
25. Dunkin GW. A possible case of congenital Johne's disease. *J Comp Pathol* 1935;48:36.

(Accepted for publication 2 February 2004)