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On farm

Ewe-Lamb Transmission Of Ovine Johne's Disease

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ABSTRACT

Knowledge of the likelihood of intrauterine or transmammary transmission of *M. a. paratuberculosis* infection in sheep is important in the design of control programs. Little has been published on this aspect of ovine Johne's disease, although studies in cattle have found up to 25% of foetuses from clinically affected cows to be infected. In this study 151 ewes from heavily infected flocks and their late term foetuses were examined using all available antemortem and necropsy tests. Five of six ewes with clinical OJD had infected foetuses. One of 54 subclinically affected ewes and none of 16 apparently uninfected ewes had infected foetuses. Only two ewes (both clinical cases which also had infected foetuses) had detectable *M. a. paratuberculosis* in their milk or mammary glands. Thus, although intrauterine or transmammary transmission may occur frequently in clinically affected sheep, it is infrequent in subclinically infected ewes or in ewes not detectably infected (even if from a heavily infected flock), suggesting that this mode of transmission is unlikely to significantly affect existing OJD control programs.

EXECUTIVE SUMMARY

Knowledge of the likelihood of intrauterine or transmammary transmission of *M. a. paratuberculosis* infection in sheep is important in the design of control programs. Little has been published on this aspect of ovine Johne's disease, although studies in cattle have found up to 25% of foetuses from clinically affected cows to be infected.

In this study 145 ewes from a heavily infected flock on Farm A, and their late term foetuses were examined. A sub-sample of 125 of the ewes had been screened for OJD between August and November 2000. At that time, blood samples were collected for AGID and IFN-γ testing, faecal samples were collected for OJD culture, and skin testing for delayed-type hypersensitivity (DTH) was performed. In August 2001, in the weeks preceding necropsy, blood samples were collected for AGID and IFN-γ, and faecal samples for OJD culture and direct PCR. At necropsy, tissue samples were collected from the ewes and their foetuses for OJD culture and histopathological examination. During other studies on Farm B, a further six pregnant sheep were necropsied after showing clinical signs suggestive of OJD, and results from these were included in the current study.

Five of six ewes with clinical OJD had infected foetuses. These results suggest that 53 – 99% of ewes with clinical and confirmed OJD could be expected to have an infected foetus. These five ewes were all in the advanced stages of OJD. In two for which full pathological results were available, severe diffuse pathology was present (one multibacillary and one with fewer organisms).

Only one of 54 subclinically affected ewes had an infected foetus. This ewe was culture positive in the uterus only, but did have clinical signs suggestive of OJD. These results suggest that 0.3 –12.5% of ewes with subclinical OJD could be expected to have an infected foetus.

None of 16 uninfected control ewes had an infected foetus.

Only two of 48 ewes (43 infected and five apparently uninfected) had *M. a. paratuberculosis* in milk or mammary tissue. These results suggest that 0.5 – 17%. of infected ewes could be expected to excrete culturally detectable *M. a. paratuberculosis* in milk.

The findings from this study are unlikely to significantly alter the current approaches taken by industry for control of OJD. Even on farms with a high prevalence of infection, the risk of intrauterine and transmammary transmission is likely to be small if suspected clinical cases of OJD are promptly culled. Such sheep are of greatest risk to the rest of the flock by their potential excretion of enormous numbers of *M. a. paratuberculosis.* The congenital risk to their own offspring is thus a minor risk for OJD transmission at the flock level. The low level of demonstrated foetal infection in subclinically affected ewes and in uninfected ewes is of more importance. Considering these two groups together, the upper 95% confidence limit for infected foetuses was just 7.5%. Remembering that only 16 uninfected ewes were included and that this is a worst case scenario in heavily infected flocks, these finding provide some assurance that congenital infection is unlikely to be a significant barrier to existing control or stud recovery programs, especially if antemortem testing is undertaken.

This project was also designed to provide samples from adult sheep with defined *M. a. paratuberculosis* infection for gamma interferon test validation, to enable CSIRO to carry out its obligations under the project OJD.025. Samples were successfully collected for testing, and culture and pathology results provided, from 145 ewes.

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1. BACKGROUND AND INDUSTRY CONTEXT

Control of ovine Johne's disease (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 for reduction of disease on individual properties, for trading from infected properties and for stud recovery programs.

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. Clinically affected sheep or cattle can excrete enormous numbers of organisms in their faeces. Sheep with multibacillary OJD were shown to excrete 10^8 organisms per gram of faeces,[54] and in cattle, levels of about 10^6 per gram have been measured.[15] If allowance is made for the effects of decontamination procedures in decreasing the numbers of organisms isolated, excretion rates for sheep of 10^{10} organisms/gram (10^{12} – 10^{13} organisms/sheep/day) are possible. Thus environmental contamination from even a single clinical case may be considerable. Moreover, *M. a. paratuberculosis* has been shown to survive for many months in the environment,[53] so contamination levels might build up over time. Doses as low as 10^3 *M. a. paratuberculosis* organisms have been shown to be infectious,[2] although recent local studies failed to infect sheep with less than 10^7 organisms.[36] Even at this higher level, faecal contamination from a single clinical case is sufficient to infect enormous numbers of susceptible animals. Exposure to these organisms originating in faeces can occur by ingestion of contaminated pasture, soil, or water, or in suckling animals, from faeces on the teats.[52]

Observational and experimental studies in cattle indicate that young animals are the most susceptible, whereas adults require higher doses and longer incubation periods to show signs of disease.[5 14 18 31 34 35 46 48] The resistance shown by adult cattle appears to be resistance to clinical disease rather than resistance to infection per se. This was shown in several experimental studies with large (180-200mg culture) oral doses of *M. a. paratuberculosis*. In one study, one month old calves had more bacilli and lesions in their tissues 5 months after dosing than 9 month old calves or adult cattle, but most of the older cattle were nonetheless infected.[18] Another study examined groups of 2 cows and 2 calves 2, 3, 4 and six months after dosing, and recovered *M. a. paratuberculosis* from 6 of the 8 calves, but from only one of 8 infected cows. However, cows examined at 2 months had more extensive lesions than the calves, while those examined later had fewer and less severe lesions, suggesting that an early host response in the cows was dealing with the infection, but nonetheless indicating that the cows had been infected.[31] Studies of natural infections reinforce the experimental findings. Four of six adult cattle exposed naturally to a heavily contaminated environment had demonstrable infection in lymph nodes without detectable histological lesions, and 3 of 6 had excreted *M. a. paratuberculosis* in the faeces, but none developed clinical disease.[35] Six of 8 bulls exposed at 16 to 27 months of age became infected, excreting the organism in faeces, but did not progress to clinical disease.[40] Less work has been published concerning age resistance in sheep, but the situation is likely to be analogous to that in cattle, with older sheep resistant to the development of clinical disease, but not necessarily to infection. Disease was produced in 8 of 9 experimentally infected lambs, 2 of 2 eight month old weaners, but in none of 8 adult ewes using an unquantified dose of intestinal material.[22] Brotherston *et al* found no difference in susceptibility as assessed by culture and histopathology 2 to 12 months after experimental infection between sheep inoculated at 3 weeks or 3 months of age (total dose about 10^6 organisms).[3 29] Later studies by the same group reported no immunity to infection up to 20 months of age (total dose about 10^8 organisms),[12] but in this latter work examinations were carried out just 2 ½ months after first infection so there was no attempt to identify whether infected animals would later clear the infection or develop disease. Similarly, recent studies with Australian merinos demonstrated that naive mature ewes were infected (as demonstrated by recovery of *M. a. paratuberculosis* from necropsy tissues in the first year post-exposure) as readily as their lambs, but whether they would progress to clinical disease was not examined.[36]

Strategies to reduce transmission can be proposed based on the above knowledge, and are designed to avoid exposure of young lambs to potentially affected adults. Such practices include artificial rearing of lambs, rearing young sheep away from adults or pasture grazed by adults, and shed rearing of rams. 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 available information specific to sheep on which to base this assumption.

There are several reports concerning possible intrauterine transmission in cattle. Most refer to foetal or uterine infection from clinically affected cows.[1 8 20 32 37 41] The rate of foetal infection from such animals is surprisingly high, with figures of 26 to 35%.[8 41] From subclinically infected cows, rates of foetal infection are about 10%.[16 44] There are only two references concerning 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 affected ewe, and *M. a. paratuberculosis* was identified in the uterine mucosa of 4 ewes, from 3 different flocks, that were reactors to the complement fixation test.[45] A report of antibodies to *M. a. paratuberculosis* in 3% of precolostral lambs from seropositive ewes is suggestive of intrauterine exposure.[28] Whether foetal infection is rarer in sheep than in cattle, or the dearth of reports is simply a reflection of less investigation is not clear. Another consideration is that most sheep strains of *M. a. paratuberculosis* have until recently resisted attempts at culture,[50] which might lead to under-reporting of foetal infection in this species, given that foetal infection is usually without lesions.[6] In summary, congenital infection of the foetus has been demonstrated frequently in cattle and at least once in sheep. Its epidemiological significance may well be underrated.

There are also a number of reports concerning 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.[42] Another found 12% of subclinically infected cows shedding in milk and that prevalence of excretion in milk correlated with the level of faecal excretion.[43] Clinically affected cows are even more likely to excrete the organism in milk, with one study showing a prevalence of 45%.[11] There are no references concerning the culture of *M. a. paratuberculosis* from the milk of sheep. However, the situation is likely to be similar to that in cattle, and a recent study using PCR detected *M. a. paratuberculosis* DNA in 88% of milk samples from sheep with positive gamma-interferon (IFN-γ) tests.[13] The numbers of organisms in infected milk may be low, although there are few references quantifying this. A single study in subclinically infected cattle found only 2-8 CFU per 50 mL.[43] If infective dose levels for *M. a. paratuberculosis* in milk are similar to those demonstrated experimentally (above), then infection via the milk may be unlikely. That the highest levels of excretion are seen in colostrum is not surprising and is probably due to its containing large numbers of macrophages.[24] Colostrum is available to the neonate at the time of highest susceptibility to infection. Moreover, antibodies to *M. a. paratuberculosis*, which may be present in colostrum of seropositive animals, have been shown experimentally to increase the uptake of *M. a. paratuberculosis* by intestinal M cells.[27] Thus, the significance of mammary excretion to the epidemiology of Johne's disease is unclear.

This project was designed to fill the gaps in knowledge concerning the occurrence of intrauterine or transmammary infection in merino sheep, so that the likelihood of such infection can be considered in the design of control programs.

2. PROJECT OBJECTIVES

- To determine whether foetuses of ewes with OJD are infected prior to birth.
- To determine whether transmission to the ovine foetus can occur in sheep with subclinical OJD.
- To correlate foetal infection with the stage of disease in the ewe.
- To determine whether *M. a. paratuberculosis* is present in the milk/colostrum of OJD infected ewes.
- To assess the risks to on-farm control of OJD due to intrauterine and transmammary transmission.
- To provide CSIRO with samples from adult sheep with defined infection for gamma interferon test validation, to enable CSIRO to carry out its obligations under the project OJD.025.

3. METHODS

3.1. Animals

The main study was conducted with sheep from Farm A, a heavily infected property near Goulburn, on which considerable previous work had been undertaken. Annual mortalities of up to 20% from OJD amongst older sheep were experienced, and seropositivity in the agar gel immunodiffusion test (AGID) amongst random groups of 50 to 133 clinically normal sheep 2-3 years of age ranged from 8 to 16%. 159 non-vaccinated, 3-year-old ewes were identified on this farm in August 2000 for possible use in this study, and 145 of these were subsequently necropsied as 4-year-olds in August 2001. These ewes had been joined over a 6-week period commencing on the 25th March 2001, and gestational ages at the time of necropsy ranged from 95-149 days. During other studies on second heavily infected farm (Farm B), a further six pregnant sheep were necropsied after showing clinical signs suggestive of OJD, and results from these were included in the current study. Results for the full range of tests were not available for all ewes from Farm B.

3.2. Sampling schedule (Farm A)

A sub-sample of 125 of the 159 identified ewes were screened for OJD between August and November 2000. Blood samples were collected for AGID and IFN-γ testing, individual faecal samples were collected for OJD culture, and skin testing for delayed-type hypersensitivity (DTH) performed.

Necropsy of the 145 ewes and their late term foetuses was performed over four farm visits between 9th and 21st August 2001. In the weeks preceding necropsy, blood samples were collected from the ewes for AGID and IFN-γ testing, and faecal samples collected for OJD culture and direct PCR. Details of samples collected at necropsy are given below. 14 of the 159 ewes identified for the study in the previous year were missing, presumed dead.

3.3. Gamma interferon (IFN-γ) assay

Blood was collected from the jugular vein into lithium heparin vacutainers, and held at room temperature for less than 12 hours prior to processing. For samples from November 2000, two 1.5 mL aliquots of well mixed blood were incubated for 18 hours at 37 ^{o}C with 100µL of Avian PPD (300 µg/mL) or phosphate buffered saline (PBS) in polystyrene cell culture plates (Costar, Corning International, New York). Plasma was collected after centrifugation at 500 g for 10 minutes, and frozen at –20 ^{o}C for three months. The samples were subsequently transported overnight at 4 ^{o}C to CSL for the enzyme immunoassay (Bovigam, Bovine gamma interferon test, CSL, Parkville, Victoria). Results were assessed using the manufacturer's recommended criteria. A response to Avian PPD was recorded if optical density (OD) (Avian PPD) was > 0.05 above that for PBS. Samples from August 2001 were collected for CSIRO's IFN-γ test evaluation. Sample stimulation and plasma collection was performed at EMAI by CSIRO staff. Chilled plasma was then transported to CSIRO, Geelong for subsequent testing. Details of these procedures, and the subsequent results and interpretations do not form part of this project.

3.4. Agar gel immunodiffusion test

Blood was collected from the jugular vein into plain vacutainers, and samples were allowed to clot and retract at room temperature, with subsequent storage at 4 ^{o}C. Serum was removed within 48 hours for testing in an AGID test. Results were recorded as negative, inconclusive, or positive (1+, 2+ or 3+).

3.5. Skin-testing for delayed hypersensitivity

Ewes were injected intradermally on the wool-free inner thigh with 0.1 mL of Avian purified protein derivative (PPD) (25,000 IU/mL, CSL, Parkville, Victoria). Skin fold thickness was measured with vernier

callipers before injection and 72 hours later, and the increase in skin-fold thickness calculated. An increase of ≥ 4mm was considered positive.

3.6. Necropsy samples

Duplicate tissue samples were collected into sterile 5 mL containers for *M. a. paratuberculosis* culture and into 10% neutral buffered formalin for histopathology.

Ewes. The following tissues were collected from each ewe:

- ileocaecal valve (ICV), 2 x terminal ileum (TI)
- 3 x ileocaecal/mesenteric lymph nodes (MLN)
- supramammary lymph node (SLN)
- milk/colostrum (for culture only, into a 10 mL sterile centrifuge tube)

Foetuses. The following tissues were collected from each foetus:

- cotyledon
- ICV, 2 x TI
- 2 x MLN
- spleen
- Blood (into a plain vacutainer for subsequent storage of serum)

For culture, the ICV and TI samples from each ewe or foetus were pooled for a single ICV/TI culture, as were the MLN samples.

3.7. Necropsy procedure, gross examination

Ewes were euthanased with intravenous pentobarbitone sodium (Lethobarb, Virbac, Australia), then placed in right lateral recumbency. Condition score (1-5) was recorded. Particular care was taken to avoid possible 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. Gloves were changed between necropsies. Ewe samples were collected in a specific order to avoid possible cross-contamination of mammary tissues from intestinal contents or MLN. The mammary gland and inguinal region were thoroughly scrubbed with medicated soap and water, to remove possible faecal contamination.

3.7.1. Removal and sampling from foetus

The flank of the ewe was incised, the uterus exteriorised and the number of foetuses recorded. Initially samples were collected from all foetuses, but subsequently, due to a very high rate of twinning, only one foetus per ewe was sampled, except when the ewe had gross lesions suggestive of OJD. The uterus was incised along the curvature of the spine of the foetus and the foetus exteriorised onto a chopping board. Blood was collected immediately from the umbilical vein(s), or heart if necessary. The umbilical cord was then cut and the foetus removed to a separate table. Foetuses were euthanased if necessary with intracardiac pentobarbitone sodium. Crown-rump measurement was recorded. A foetal cotyledon was collected from the placenta immediately after the removal of the foetus. The abdomen of the foetus was opened to allow visualisation of the abdominal contents. MLN, ICV and TI samples were collected as below for ewes, and the entire spleen was collected. Samples from foetuses were later selected for

culture and histopathological examination based on the ewe findings. Samples from all foetuses collected from infected ewes were processed, and samples from the foetuses of a further 16 randomly selected uninfected ewes from Farm A were also processed.

3.7.2. Ewe sampling

After foetus removal and cotyledon sampling, an incision was made 2-3cm above the base of the teat, and up to 10 mL of mammary secretion was collected. Rarely was the secretion sufficiently fluid for easy collection using a sterile syringe. Frequently it had a thick honey-like consistency and a sterile needle cap was used to scoop out at much as practical. Many sample sizes were less than 1 mL in volume. SLN was then collected. Finally the intestines were examined. The caecum was exteriorised, then pulled cranially to display the ICV, TI, MLN and jejunal loops. Gross lesions of OJD were assessed visually and by palpation. Any thickening of TI/ICV, enlargement of MLN or cording of lymph vessels was recorded. MLN samples were then collected, taking the ileocaecal node, caudal jejunal node and another more proximal node. The whole ICV was then sampled (with some attached TI), followed by two more TI samples at intervals of approximately 10cm proximal from the ICV.

3.8. Histopathology

Fixed tissues were processed routinely for histopathology, embedded in paraffin and 5μm sections cut and stained with hematoxylin and eosin (HE) and Ziehl-Neelsen (ZN). Slides were examined by light microscopy. The lesions from ewes were graded using two different systems. Intestinal and node lesions were assessed using a 1-7 scale for intestines, and 1-3 scale for nodes (Marshall, unpublished). Intestinal lesions were also graded using an adaptation of the classification of Perez[33] to include lesions in the MLN. Details of these classification systems are given in Appendix 1.

3.9. Culture for M. a. paratuberculosis

Freshly collected samples were held in an esky at approximately 4 ^{o}C, then transferred to a -80°C freezer within 10 hours. They were held at -80 ^{o}C until prepared for Bactec culture.

3.9.1. Preparation of tissues

These were prepared as previously described.[50 51] Briefly, each tissue sample (2-5 gm) was trimmed of excess fat, homogenised in 2 mL of sterile normal saline, then mixed with 25 mL of 0.75% HPC in a 35 mL polystyrene tube. This was left undisturbed at room temperature for 72 hours. A 100μL aliquot was then carefully 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.

3.9.2. Preparation of faeces

A method [51] based on the double incubation method of Whitlock and Rosenberger [49] was used. Briefly, each faecal sample (2-5 gm) was mixed with 10-12 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. A 5 mL aliquot of the surface fluid was transferred to a 35 mL polystyrene tube containing 25 mL of 0.9% hexadecylpyridinium chloride (HPC) (Sigma Chemical Co., St Louis, Mo) in half-strength brain heart infusion broth (BHI) (Oxoid, Basingstoke, England) and allowed to stand at 37 ^{o}C for 24 h. The tube was then centrifuged at 900 X g for 30 mins. 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) (VAN) and incubated for 72 h at 37 ^{o}C. Sediment was then resuspended by vigorous agitation, and a 100 μL aliquot was inoculated into a Bactec vial.

3.9.3. Preparation of mammary secretion

Samples were thawed overnight at 4°C. They were then transferred to a new 10 mL sterile centrifuge tube. Sterile phosphate buffered saline (PBS) was added as necessary to make the volume up to 10 mL.

Samples were then mixed on a tube rotor at 37°C for 2-3 hrs, then centrifuged at 2500 x *g* for 15 minutes. The cream and whey layers (supernatant) were discarded and 5 mL HPC was added to the pellet. The samples were again mixed on a tube rotor at 37°C for 1-2 hrs to resuspend the pellets. The resuspended material was left undisturbed at room temperature for 72 hrs. A 100μL aliquot was then carefully 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.

3.9.4. Bactec culture

Bactec vials were incubated at 37 ^{o}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.[50] Growth indices (GI) were measured weekly with an automatic ion chamber (Bactec 460; Johnston Laboratories, Towson, Md). PCR for IS*900* and restriction endonuclease analysis (REA) were performed on material from GI positive vials to confirm that the observed GI were due to *M. a.* subsp. *paratuberculosis* [7 51].

3.10. Direct PCR on faeces

This was done as previously described.[21] Briefly, 200mg of faeces was mixed with 700 μL PBS in an Eppendorf tube. To extract the DNA, the tube was heated at 55 ^{o}C for 30 min, then vortexed for 3 min, before boiling at 105 ^{o}C for 30 min. Tubes were then centrifuged at 12,500g for 5 min. DNA was then purified from 300 μL of the supernatant using a resin based method (Promega-Wizard™ PCR Preps DNA purification system – Cat No. A7170). 5 μL of the extracted DNA was used in an IS*900* PCR reaction, with forward primer IS*900*/150C[26] and reverse primer P91,[47] followed by REA.

3.11. Statistical analysis, classification of infected animals

The 95% confidence limits for the percentage of ewes with infected foetuses were obtained from tables of binomial confidence limits.[4] Because of the lack of accepted "gold standards" for OJD, the following criteria for classification of sheep with regard to OJD status were used:

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

Subclinical case. Infected sheep without clinical signs of OJD (emaciation with or without diarrhoea) were classified as subclinical OJD cases. Infected sheep with clinical signs, but in which infection and lesions were not sufficiently extensive to have caused the clinical signs were also classified as subclinical cases (the clinical signs mimicking OJD presumably caused by some other unidentified problem).

Clinical case. Infected sheep with clinical signs of OJD were classified as confirmed clinical cases only if the pathology and/or tissue infection was widespread and severe.

Uninfected sheep. These animals were histopathologically and culturally negative for OJD. They may have had other evidence for OJD exposure (immunological responses). There were also several sheep which had clinical signs suggestive of OJD, but which were uninfected.

4. RESULTS

4.1. Ewes

4.1.1. Antemortem testing

Details of all antemortem tests, both immediately prior to necropsy and 12 months previously are given in Appendix 2. On Farm A, sheep that were emaciated (condition score 1) were considered to have clinical signs suspicious for OJD.

Based on clinical signs and the 2001 results for faecal culture and gel testing (ie currently available, routinely applied tests) only 26 sheep would have been classified as definitely or possibly infected. Six of these sheep would have been falsely classified as infected – in these the only suspicion for OJD was poor condition, and all other tests later were negative. On the other hand 40 subclinically affected ewes remained undetected. If the results of 2001 DPCR, and the DTH, IFN-γ and faecal culture results from 2000 are considered also (ie exhaustive antemortem testing), a total of 60 sheep would have been considered to be possibly infected, with 26 false positives, and 26 false negatives. For the purposes of this project, equivocal or trace reactions were included as positive results. Results for the 2001 IFN-γ testing were not available for this report, and it is highly likely that the inclusion of this test in the antemortem assessments would have increased the number of subclinically infected sheep detected.

Interestingly, of the 14 ewes which were missing at the August 2001 sampling, 11 had been faecal culture positive for *M. a. paratuberculosis* at the previous examination, and it is highly likely that death was due to OJD.

4.1.2. Culture results

Full results are given in Appendix 3.

A total of 52 out of 151 (34%) ewes had at least one positive tissue or faeces culture. Two ewes only from 141 were positive in the SLN. In both these animals no other tissue was culture positive and there were no lesions consistent with OJD. Only one ewe (6044) had a positive faecal culture in the absence of confirmation by tissue culture. This animal was faecal culture positive at the 2000 sampling, but negative to all tests a year later, suggesting either recovery or passive excretion.

Milk and/or mammary gland samples from 48 ewes were cultured. 43 had other cultural or pathological evidence for OJD infection and 5 were apparently uninfected. Only two positive cultures were obtained, both from the mammary tissue of sheep with severe clinical confirmed OJD.

4.1.3. Pathological findings

Full results are give in Appendix 4.

36 of 145 (25%) had histopathological lesions indicative of OJD. All but eight of these were also culture positive. These eight ewes all had focal lesions only in the PP of the intestine or MLN, and in five of these, small numbers of acid-fast organisms were detected.

4.2. Foetuses

62 foetuses from 53 of 56 culture and/or histologically positive ewes were examined (included 8 sets of twins and one of triplets). A further 18 foetuses from 16 negative ewes were examined as controls. Culture results only were available from the 6 additional foetuses from Farm B.

4.2.1. Culture results

Full results are included in Appendix 3. Overall, *M. a. paratuberculosis* was isolated from foetal tissues of only six out of 86 foetuses. In every case *M. a. paratuberculosis* infection was confirmed in the ewe. Foetal cotyledon was positive in every case, and in four of the six foetuses was the only culture positive foetal tissue. In the other two foetuses *M. a. paratuberculosis* also isolated from other tissues, namely spleen, liver and umbilicus.

4.2.2. Histopathology

There were no significant lesions in any of the foetal tissues examined.

4.3. Overview and correlation of infection status in ewe and foetus

Tables 1 & 2 illustrate the percentages of infected foetuses obtained from ewes of differing status. A detailed overview of summary results for all tests in individual ewes and foetuses is given in Appendix 5. In total, 60 ewes were identified as infected (based on positive histopathology or positive culture), and from these sheep, six infected foetuses were detected out of 68 foetuses examined.

Table 1. Cross tabulation of foetal infection with antemortem status of ewe[1]

	Number of sheep	Number truly infected	Foetal infection (%)
Positive	26	20	23
Negative	125	40	0

[1] Based on clinical suspicion, AGID, and faecal culture (ie routine antemortem testing)

Table 2. Cross tabulation of foetal infection with true infection status of ewe[1]

	Number of sheep	Foetal infection (%)
Clinical OJD	6	83
Subclinical infection	54	2
Uninfected	91	0[2]

[1] Classified as infected if positive on culture and/or histopathology

[2] Foetuses from only 16 of the uninfected ewes were actually cultured

Only seven ewes in this study had clinical signs consistent with OJD and were later confirmed by culture and/or histopathology to be infected. One of these ewes was culture positive only in the uterus, and the observed clinical signs were thus considered to have been unrelated. It was therefore classified as a subclinical case, leaving only six confirmed clinical OJD ewes in this study. Five of these (83%) had an infected foetus. Although a very small sample, the results are unequivocal (95% confidence interval approximately 53 – 99%). However, foetuses from only two of these ewes (28%) had infection in tissues other than cotyledon.

From 54 subclinically infected ewes, there were 58 uninfected foetuses, and a single infected foetus (this from the doubtful clinical case above). The 95% confidence limits for infected foetuses from subclinically infected ewes are 0.3 – 12.5%.

From 16 uninfected control ewes there were 18 uninfected foetuses.

Two of 43 infected ewes had detectable *M. a. paratuberculosis* in milk or mammary tissue (95% confidence interval 0.5 – 17%). Both had severe clinical and confirmed OJD, and both had infected foetuses. No positive cultures were obtained from 5 uninfected ewes. A further two ewes had infection in the SLN, without other findings suggestive of OJD.

5. DISCUSSION

This study was a "worst case scenario" conducted in flocks with a high prevalence of OJD. Even so, only six of a total of 86 foetuses were demonstrated to be infected. In four of these, the only culture positive foetal tissue was cotyledon, and this sample would almost certainly have contained some maternal tissue. This leaves only two foetuses with unequivocal foetal infection. Five of the six infected foetuses were from ewes with confirmed clinical OJD. Only one of 69 subclinically infected or uninfected ewes yielded an infected foetus. Considering these together, we can be 95% confident that less than 7.5% of such sheep (non-clinical cases from a heavily infected flock) might have an infected foetus.

Of the six ewes with infected foetuses, two were from Farm A and had received the full battery of tests. Both these sheep had clinical signs consistent with OJD (body condition score of 1), both were faecal culture positive and DPCR positive, and one was positive on the gel test. Neither would have been missed with routine antemortem screening for OJD. At necropsy, both had typical gross lesions of OJD and both had severe diffuse histopathological lesions, one multibacillary and one with fewer organisms.

The remaining four infected foetuses were from ewes from Farm B, all with clinical signs suggestive of OJD. No pathology results were available, and faecal culture results were available for only two (both positive). Three of the four were culture positive in multiple gut and MLN samples, as well as uterus or uterine lymph node, and it is likely that they were indeed severe clinical OJD cases, and as such should readily have been detected by antemortem testing. The two foetuses with generalised *M. a. paratuberculosis* infection and one with infection detected only in cotyledon were from these diseased ewes. The final ewe, the foetus from which was infected only in cotyledon, was culture positive only in the uterus. Gut and MLN samples were culture negative, although segmental lesions may have been missed in the necropsy sampling. Whether OJD was responsible for the clinical condition of this ewe is thus uncertain, and a note of caution remains concerning the possibility of an infected foetus deriving from a subclinically affected ewe.

None of the milk samples were culture positive, although *M. a. paratuberculosis* was isolated from the mammary glands of two ewes from Farm B (both clinical cases). A further two ewes were culture positive in the SLN with no other findings suggestive of OJD, but the significance of this is unclear. The milk samples in this study were not ideal. Due to the range of gestational ages, samples ranged from gelatinous secretion from a previous lactation through to colostrum. The volume which could be collected varied from less than 1 mL up to 10 mL, and the consistency of the samples was extremely varied. The culture method for milk was a compromise based on published reports and the routine EMAI tissue protocol. It probably had an analytical sensitivity of 10^2 – 10^3 organisms.[36] The combination of small sample volume and culture sensitivity means that small concentrations of organisms would not have been detected. In reported cattle studies, 50mL samples were used and the numbers of organisms isolated were low (2-8 CFU per 50 mL).[43] Such levels would not have been detected in the current study. Another possible limitation in the culture technique of the current study was the use only of the pellet after centrifugation. One previous study in cattle suggested that at least some *M. a. paratuberculosis* organisms separated into the cream layer,[25] but the nature of the samples in the current study made consistent collection of a cream layer impractical. However, even allowing for the cultural limitations, if infective dose levels of *M. a. paratuberculosis* in milk for sheep are similar to those demonstrated experimentally (see introduction), then infection via the milk is unlikely to be of practical significance. In the only two ewes with positive cultures from milk or mammary tissue, foetuses were culture positive anyway. Further studies, using samples from a lactating mob, would be needed to better determine the rate of transmammary shedding in sheep, should such information be desired.

The use of pooled colostrum in calves could potentially disseminate infection rapidly in a dairy herd. Note also that pasteurisation does not completely remove *M. a. paratuberculosis* from colostrum.[24] However, in beef or sheep enterprises where neonates usually remain with their dams there would be significant risk of congenital infection and/or concurrent oral exposure to infective faeces, so whether or not infection occurs via the milk has fewer practical implications. In the special case, where small numbers of genetically valuable lambs are to be "salvaged", colostrum could be sourced from tested uninfected sources and so completely avoid any risk of transmammary infection. On the other hand, possible congenital infection could never be completely avoided, but the results from the present study indicate that simply excluding clinically affected ewes (including ewes in poor condition) will greatly reduce the risk of foetal infection. Antemortem testing could be expected to further reduce risk. Note, however, that even exhaustive antemortem testing will fail to detect a large number of subclinically infected sheep.

The possibility of transmission by other reproductive routes (venereal, in semen by AI, and by embryo transfer) should also be considered when stud salvage operations are planned. All three routes have been shown to be possible in cattle, but their practical significance is probably limited. *M. a. paratuberculosis* has been isolated from the semen of clinically affected bulls[17] and rams,[10] and from the uterine fluids of clinically affected cows.[38] Thus, venereal transmission in either direction is also theoretically possible, as is direct infection of a developing embryo, without established infection in the dam. *M. a. paratuberculosis* has also been isolated from uterine flush fluids from clinically infected cows,[38] and from washed ova[39] indicating that embryo transfer from infected animals to uninfected donors is not entirely without risk (both to the developing embryo and the recipient dam). However, these possible routes of infection are probably of minimal significance in the field. Clearance of the organism from the uteri of cows after intra-uterine inoculation of high doses of *M. a. paratuberculosis* has been demonstrated,[23] and infected males are likely to shed large numbers of organisms in faeces before significant numbers appear in the semen.[19]

In all the reported studies of foetal *M. a. paratuberculosis* infection, and also in the current study, there is no indication whether any of the infected foetuses may later become clinical cases, or even subclinical carriers. The ovine foetus has been reported to be unable to mount an immune response to certain antigens including BCG,[30] (p513) and it is possible that this immaturity of the immune system extends also 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 a 1935 observation that a calf born to a cow with clinical JD 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.[9]

6. SUCCESS IN ACHIEVING OBJECTIVES

This study successfully achieved its objectives, summarised below:

- **To determine whether foetuses of ewes with OJD are infected prior to birth.**

The results from this study indicated that 53 – 99% of ewes with clinical and confirmed OJD could be expected to have an infected foetus.

- **To determine whether transmission to the ovine foetus can occur in sheep with subclinical OJD.**

The results from this study indicated that 0.3 – 12.5% of ewes with subclinical OJD could be expected to have an infected foetus.

- **To correlate foetal infection with the stage of disease in the ewe.**

Five of the six ewes with infected foetuses were in the advanced stages of OJD. In the two for which full pathological results were available, severe diffuse pathology was present (one multibacillary and one with fewer organisms). The single ewe classified as a subclinical case with an infected foetus was culture positive in the uterus only, but did have clinical signs suggestive of OJD.

- **To determine whether *M. a. paratuberculosis* is present in the milk/colostrum of OJD infected ewes.**

The results from this study indicated that 0.5 – 17% of infected ewes could be expected to have culturally detectable *M. a. paratuberculosis* in milk or mammary tissue.

- **To assess the risks to on-farm control of OJD due to intrauterine and transmammary transmission.**

Even on farms with a high prevalence of infection, the risk of intrauterine and transmammary transmission is likely to be small if suspected clinical cases of OJD (including sheep in emaciated condition) are promptly culled. In stud recovery programs, rigorous antemortem testing should further reduce risk.

- **To provide CSIRO with samples from adult sheep with defined infection for gamma interferon test validation, to enable CSIRO to carry out its obligations under the project OJD.025.**

Samples were successfully collected for testing, and culture and pathology results provided, from 145 ewes from Farm A.

7. IMPACT ON MEAT AND LIVESTOCK INDUSTRY

These findings are unlikely to significantly alter the approaches taken by industry for control of OJD.

The finding of high levels of foetal infection in ewes with clinical OJD is interesting, but not unexpected considering the published studies in cattle. This does highlight, however, the need for good stock management and the culling of clinically affected sheep. Such sheep are of greatest risk to the rest of the flock by their potential excretion of enormous numbers of *M. a. paratuberculosis.* The congenital risk to their own offspring (which may not survive anyway due to inability of the ewe to care for them) is thus a minor risk for OJD transmission at the flock level.

The low level of demonstrated foetal infection in subclinically affected ewes and in uninfected ewes is of more importance. Considering these two groups together, the upper 95% confidence limit for infected foetuses was just 7.5%. Remembering that only 16 uninfected ewes were included and that this is a worst case scenario in heavily infected flocks, these findings provide some assurance that congenital infection is unlikely to be a significant barrier to existing control or stud recovery programs, especially if antemortem testing is undertaken.

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APPENDICES

Appendix 1**. Histopathological classification systems for OJD**

Appendix 2**. Antemortem assessment of ewes**

Appendix 3**. Culture results (ewes and foetuses)**

Appendix 4**. Pathological findings (ewes)**

Appendix 5**. Overview of summary results (ewes and foetuses)**

Appendix 1. Histopathological classification systems

1. Marshall (unpublished) seven point system:

Intestinal lesion severity (H & E stain)

0	No lesion
1	Suspicious
2	Slight - small focal
3	Mild - small lesions multifocal
4	Mild to moderate - larger clusters multifocal
5	Moderate - multifocal coalescing (half the lamina propria)
6	Moderate to severe - diffuse (not all the lamina propria)
7	Severe - sever diffuse (most of lamina propria)

Lymph node lesion severity (H & E stain)

0	No lesion
1	Mild (small focal lesions)
2	Moderate (larger lesions multifocal)
3	Severe (diffuse)

Ziehl-Neelsen stain (intestine or node)

0	No acid-fast organisms
1	Individual or small numbers, limited foci
2	Small numbers, multiple foci
3	Moderate numbers, diffuse
4	Large numbers, diffuse

2. Adapted from Perez:

0	No lesion
1	Focal lesions, confined to PP
2	Focal lesions, involving PP and adjacent mucosa
2n	Focal lesions, involving MLN, but no intestinal lesions
3a	Multifocal lesions, involving PP, adjacent and remote mucosa
3b	Diffuse lesions, multibacillary
3c	Diffuse lesions, paucibacillary

Appendix 2. Antemortem assessment of ewes

		Sampling August 2000[1]				Sampling August 2001[2]					Summary results	
Source	Tag no.	AGID	Faecal culture	DTH	IFN[3]	Condition score	Clinical signs	AGID	Faecal culture	DPCR	Routine tests[4]	Exhaustive tests[5]
Hayes	1088	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	1701	-	-	2	0.01	2	-	-	-	-	-	-
Hayes	1702	-	-	2.8	0.02	2	-	-	-	-	-	-
Hayes	1703	-	-	2	0.005	3	-	-	-	-	-	-
Hayes	1705	-	-	9.3	0.12	3	-	-	-	-	-	+
Hayes	1706	-	-	1.6	0.089	2	-	-	-	-	-	+
Hayes	1707	-	c	0.7	0.001	2	-	-	-	-	-	-
Hayes	1708	-	-	2.8	0.014	2	-	-	-	-	-	-
Hayes	1709	-	-	2.6	-0.002	3	-	-	-	-	-	-
Hayes	1710	-	-	2.8	0.019	2	-	-	c	-	-	-
Hayes	1711	-	-	2.9	0.023	2	-	-	-	-	-	-
Hayes	1712	-	-	2.4	0.27	2	-	inc	-	-	+	+
Hayes	1713	-	-	3	0.007	3	-	-	-	-	-	
Hayes	1714	-	-	2	0.01	3	-	-	-	-	-	
Hayes	1715	-	-	5.5	0.054	3	-	inc	+	2+	+	+
Hayes	1716	-	-	2.5	0.216	3	-	-	-	-	-	+
Hayes	1717	-	-	5.7	0.038	2	-	-	-	-	-	+
Hayes	1718	-	-	2.9	-0.002	4	-	-	-	-	-	
Hayes	1719	-	-	3.5	0.01	3	-	-	-	-	-	
Hayes	1720	-	-	5.1	0.018	3	-	-	-	-	-	+
Hayes	1721	-	-	3	0.031	3	-	-	-	-	-	
Hayes	1723	-	-	3.2	0.058	3	-	-	-	-	-	+
Hayes	1724	-	-	9.1	0.01	3	-	-	-	-	-	+
Hayes	1725	-	-	5.9	0.017	2	-	-	-	-	-	+
Hayes	2062	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2063	ns	ns	ns	ns	2	-	-	-	-	-	-
Hayes	2064	ns	ns	ns	ns	1	+	-	-	-	+	+
Hayes	2065	ns	ns	ns	ns	2	-	-	-	-	-	-
Hayes	2066	ns	ns	ns	ns	2	-	-	-	-	-	-
Hayes	2067	ns	ns	ns	ns	2	-	-	-	-	-	-
Hayes	2068	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2069	ns	ns	ns	ns	2	-	-	c	tr	-	+
Hayes	2070	ns	ns	ns	ns	2	-	-	-	-	-	-
Hayes	2071	ns	ns	ns	ns	2	-	-	-	-	-	-
Hayes	2072	ns	ns	ns	ns	2	-	-	-	-	-	-
Hayes	2073	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2074	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2075	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2076	ns	ns	ns	ns	na	-	-	-	-	-	-
Hayes	2077	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2078	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2079	ns	ns	ns	ns	2	-	-	-	-	-	-
Hayes	2080	ns	ns	ns	ns	3	-	-	c	tr	-	+
Hayes	2081	ns	ns	ns	ns	2	-	2+	-	-	+	+

Appendix 2. Antemortem assessment of ewes

		Sampling August 2000[1]				Sampling August 2001[2]					Summary results	
Source	Tag no.	AGID	Faecal culture	DTH	IFN[3]	Condition score	Clinical signs	AGID	Faecal culture	DPCR	Routine tests[4]	Exhaustive tests[5]
Hayes	2082	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2083	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2084	ns	ns	ns	ns	2	-	-	-	-	-	-
Hayes	2085	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2086	ns	ns	ns	ns	2	-	-	-	-	-	-
Hayes	2087	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2089	ns	ns	ns	ns	3	-	-	c	-	-	-
Hayes	2090	ns	ns	ns	ns	3	-	2+	-	-	+	+
Hayes	2091	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2092	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2093	ns	ns	ns	ns	3	-	-	-	-	-	-
Hayes	2094	ns	ns	ns	ns	1	+	-	c	-	+	+
Hayes	2096	ns	ns	ns	ns	4	-	-	-	-	-	-
Hayes	6002	-	-	9	0.102	1	+	-	+	3+	+	+
Hayes	6003	-	-	2.3	0.033	4	-	-	-	-	-	-
Hayes	6004	-	-	4.6	0.054	2	-	-	-	-	-	+
Hayes	6005	-	-	2.4	0.011	3	-	-	c	-	-	-
Hayes	6006	-	-	3.1	0.121	2	-	-	-	-	-	+
Hayes	6007	-	-	3	0.206	2	-	-	-	-	-	+
Hayes	6008	-	-	4	0.003	3	-	-	-	-	-	+
Hayes	6009	-	-	2	0.01	3	-	-	-	-	-	-
Hayes	6010	-	-	2.9	0.016	1	+	-	-	-	+	+
Hayes	6011	-	-	0.9	0.015	2	-	-	-	-	-	-
Hayes	6012	-	-	2.6	0.022	3	-	-	-	-	-	-
Hayes	6013	-	-	5.8	0.027	3	-	-	-	-	-	+
Hayes	6014	-	-	4.4	0.035	3	-	-	-	-	-	+
Hayes	6015	-	-	2.5	-0.014	3	-	-	c	-	-	-
Hayes	6016	-	-	2	-0.001	3	-	-	+	2+	+	+
Hayes	6017	-	-	2	0.105	3	-	-	+	-	+	+
Hayes	6018	-	-	2	0.008	3	-	-	c	-	-	-
Hayes	6019	-	-	1.3	0.008	3	-	-	-	-	-	-
Hayes	6020	-	-	4.3	0.027	2	-	-	-	1+	+	+
Hayes	6021	-	-	3.1	0.036	3	-	-	-	ns	-	-
Hayes	6022	-	+	3.9	0.526	1	+	1+	+	1+	+	+
Hayes	6023	-	-	1.6	0.01	2	-	-	c	tr	-	+
Hayes	6024	-	-	8.9	2.219	2	-	1+	-	-	+	+
Hayes	6025	-	-	0.7	0.004	2	-	-	-	-	-	-
Hayes	6026	-	-	2.6	0.027	4	-	-	c	-	-	-
Hayes	6029	-	-	1.3	0.012	3	-	-	c	-	-	-
Hayes	6030	-	-	2.3	0.016	3	-	-	-	-	-	-
Hayes	6031	-	-	1.4	0	3	-	-	-	-	-	-
Hayes	6032	-	-	11.3	0.017	3	-	-	-	-	-	+
Hayes	6033	-	-	2.9	0.048	3	-	-	-	-	-	-
Hayes	6034	-	-	3.4	0.014	2	-	-	-	-	-	-

Appendix 2. Antemortem assessment of ewes

		Sampling August 2000[1]				Sampling August 2001[2]					Summary results	
Source	Tag no.	AGID	Faecal culture	DTH	IFN[3]	Condition score	Clinical signs	AGID	Faecal culture	DPCR	Routine tests[4]	Exhaustive tests[5]
Hayes	6035	-	-	1.9	0.01	3	-	-	c	-	-	-
Hayes	6036	-	-	1.9	0.023	3	-	-	+	-	+	+
Hayes	6037	-	-	9.2	0.596	3	-	-	-	-	-	+
Hayes	6038	-	-	3.9	0.018	2	-	-	-	-	-	-
Hayes	6040	-	-	2	0.01	2	-	-	-	-	-	-
Hayes	6041	inc	-	2	0.111	3	-	-	-	-	-	-
Hayes	6044	-	+	0.4	0.024	3	-	-	-	-	-	+
Hayes	6046	-	-	2.4	0.027	2	-	-	-	-	-	-
Hayes	6047	-	-	4	0.01	3	-	-	-	-	-	-
Hayes	6048	-	-	6.4	0.03	3	-	-	-	-	-	+
Hayes	6049	-	-	2	0.02	3	-	-	-	-	-	-
Hayes	6050	-	-	4	0.12	2	-	-	-	-	-	+
Hayes	6051	-	-	0.8	0.029	3	-	-	-	-	-	-
Hayes	6052	-	-	2.6	0.023	3	-	-	-	-	-	-
Hayes	6054	-	-	1.1	0.038	3	-	-	-	-	-	-
Hayes	6055	-	-	4.2	0.009	1	+	-	-	-	+	+
Hayes	6056	-	-	0.3	0.014	3	-	-	-	-	-	-
Hayes	6057	-	-	6	0.029	3	-	-	-	-	-	-
Hayes	6058	-	-	1.6	0.032	3	-	-	-	-	-	-
Hayes	6059	-	-	4.1	0.035	3	-	-	-	-	-	+
Hayes	6060	-	-	0.4	0.034	3	-	-	-	-	-	-
Hayes	6061	-	-	8.8	0.413	2	-	1+	-	-	+	+
Hayes	6063	-	-	3.2	0.057	4	-	-	-	-	-	+
Hayes	6064	-	-	5.7	0.052	3	-	-	-	-	-	+
Hayes	6065	-	-	2.1	0.017	2	-	-	-	-	-	-
Hayes	6066	-	-	2.9	0.169	3	-	-	-	-	-	+
Hayes	6067	-	-	2.7	0.012	3	-	-	-	-	-	-
Hayes	6068	-	-	3.7	-0.027	3	-	-	-	-	-	-
Hayes	6069	-	-	2	-0.001	3	-	-	-	-	-	-
Hayes	6070	-	-	2.5	0.014	2	-	-	-	-	-	-
Hayes	6071	-	-	0.6	-0.002	2	-	-	c	-	-	-
Hayes	6072	-	-	1.5	-0.02	2	-	-	-	-	-	-
Hayes	6074	-	+	3.3	0.301	3	-	-	+	-	+	+
Hayes	6075	-	-	3.6	-0.002	2	-	-	-	ns	-	-
Hayes	6077	-	-	2.1	-0.02	4	-	-	-	-	-	-
Hayes	6078	-	-	3.7	0.013	3	-	-	-	-	-	-
Hayes	6079	-	-	7.9	0.127	2	-	-	-	-	-	+
Hayes	6080	-	-	1.6	-0.013	2	-	-	-	-	-	-
Hayes	6081	-	-	3.6	0.134	2	-	-	-	-	-	+
Hayes	6082	-	-	1.6	0.001	3	-	-	-	-	-	-
Hayes	6083	-	-	1.4	0.011	3	-	-	-	-	-	-
Hayes	6084	-	-	1.2	0.021	3	-	-	-	-	-	-
Hayes	6085	-	-	5.6	0.079	3	-	-	-	-	-	+
Hayes	6086	-	-	4.1	0.064	2	-	-	-	-	-	+

Appendix 2. Antemortem assessment of ewes

		Sampling August 2000[1]				Sampling August 2001[2]					Summary results	
Source	Tag no.	AGID	Faecal culture	DTH	IFN[3]	Condition score	Clinical signs	AGID	Faecal culture	DPCR	Routine tests[4]	Exhaustive tests[5]
Hayes	**6087**	-	-	1.6	-0.033	2	-	-	-	-	-	-
Hayes	**6088**	-	-	2.1	0.006	3	-	-	-	-	-	-
Hayes	**6089**	-	-	3.7	0.098	2	-	-	+	3+	+	+
Hayes	**6090**	-	-	2.3	0.017	2	-	-	c	-	-	-
Hayes	**6091**	-	-	2.7	0.059	2	-	-	-	-	-	+
Hayes	**6092**	-	-	2.1	0.019	2	-	-	-	ns	-	-
Hayes	**6093**	-	-	3.1	0.055	2	-	-	-	-	-	+
Hayes	**6094**	-	-	1	0.016	2	-	-	-	-	-	-
Hayes	**6096**	-	-	3.4	0.056	3	-	-	-	tr	-	+
Hayes	**6097**	-	-	9	0.035	3	-	-	-	-	-	+
Hayes	**6098**	-	+	2.1	0.118	3	-	-	+	-	+	+
Hayes	**6099**	-	-	1	0.017	3	-	-	-	-	-	-
Hayes	**6100**	1+	-	8.4	0.199	3	-	1+	-	-	+	+
S. Uni	**P1**	ns	ns	ns	ns		+		+	nd	+	+
S. Uni	**P**	ns	ns	ns	ns		+		-	nd	+	+
S. Uni	**O**	ns	ns	ns	ns		+		-	nd	+	+
S. Uni	**NT**	ns	ns	ns	ns		+		-	nd	+	+
S. Uni	**B1**	ns	ns	ns	ns		+		ns	nd	+	+
S. Uni	**B**	ns	ns	ns	ns		+		ns	nd	+	+

Indicates a positive or equivocal result

[1] 14 sheep tested in Aug 2000 had died by Aug 2001, and are not listed. 11/14 were faecal culture positive

[2] Results for IFN and Elisa (not available for this report) are included in Project OJD.025 (CSIRO)

[3] Results for stimulation with avian PPD, positive if OD (avian) exceeds OD (PBS) by > 0.05.

[4] Includes clinical signs, faecal culture and gel test results from 2001 only

[5] Includes all 2001 and 2000 results

Appendix 3. Culture results (ewes and foetuses)

Sheep details		Ewe results								Foetus results						
Source	Tag no.	Faeces 2000	Faeces	MLN	Ileum/ICV	Suprmammary lymph node	Milk/udder	Other	Ewe summary	MLN	Ileum/ICV	Spleen	Cotyledon	Other	Foetus summa	Comment
Hayes	1088	ns	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	1701	-	-	-	-	-	ns		-							
Hayes	1702	-	-	-	-	-	ns		-							
Hayes	1703	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	1705	-	-	-	c	-	ns		-	-	-	-	-		-	
Hayes	1706	-	-	-	-	-	ns		-							
Hayes	1707	c	-	-	-	-	ns		-							
Hayes	1708	-	-	+ 5	-	-	ns		+	-	-	-	-		-	
Hayes	1709	-	-	-	-	-	ns		-							
Hayes	1710	-	c	-	-	-	ns		-	-	-	-	-		-	
Hayes	1711	-	-	-	+ 11	-	-		+	-	-	-	-		-	
Hayes	1712	-	-	-	+ 7	-	-		+	-	-	-	-		-	
Hayes	1713	-	-	-	-	-	ns		-							
Hayes	1714	-	-	-	-	-	ns		-							
Hayes	1715	-	+ 6	+ 6	+ 3	-	-		+	-	-	-	-		-	
Hayes	1716	-	-	-	c	-	ns		-	-	-	-	-		-	Twins (both -ve)
Hayes	1717	-	-	+ 7	c	-	-		+	-	-	-	-		-	
Hayes	1718	-	-	-	-	-	ns		-							
Hayes	1719	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	1720	-	-	-	-	-	ns		-							
Hayes	1721	-	-	-	+ 7	-	ns		+	-	-	-	-		-	
Hayes	1723	-	-	-	+ 10	-	-		+	-	-	-	-		-	
Hayes	1724	-	-	-	-	-	ns		-							
Hayes	1725	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	2062	ns	-	-	-	-	ns		-							
Hayes	2063	ns	-	-	-	-	ns		-							
Hayes	2064	ns	-	-	-	-	ns		-							
Hayes	2065	ns	-	-	-	+ 12	-		+	-	-	-	-		-	Twins (both -ve)
Hayes	2066	ns	-	-	-	-	ns		-							
Hayes	2067	ns	-	-	-	-	ns		-							
Hayes	2068	ns	-	-	-	-	ns		-							

Appendix 3. Culture results (ewes and foetuses)

Sheep details		Ewe results								Foetus results						
Source	Tag no.	Faeces 2000	Faeces	MLN	Ileum/ICV	Suprmammary lymph node	Milk/udder	Other	Ewe summary	MLN	Ileum/ICV	Spleen	Cotyledon	Other	Foetus summa	Comment
Hayes	2069	ns	c	-	-	-	ns		-							
Hayes	2070	ns	-	-	c	-	ns		-	c	-	-	-		-	Twins (both -ve)
Hayes	2071	ns	-	-	+ 6	-	-		+	-	-	-	-		-	
Hayes	2072	ns	-	-	+ 5	-	-		+	-	-	-	-		-	
Hayes	2073	ns	-	-	-	ns	ns		-							
Hayes	2074	ns	-	-	-	-	ns		-							
Hayes	2075	ns	-	-	-	-	-		-	-	-	-	-		-	
Hayes	2076	ns	-	-	-	-	ns		-							
Hayes	2077	ns	-	-	-	-	ns		-							
Hayes	2078	ns	-	-	-	-	ns		-							
Hayes	2079	ns	-	-	c	-	ns		-							
Hayes	2080	ns	c	-	-	-	ns		-							
Hayes	2081	ns	-	-	-	-	ns		-							
Hayes	2082	ns	-	-	-	-	ns		-							
Hayes	2083	ns	-	-	-	-	ns		-							
Hayes	2084	ns	-	-	-	+ 20	ns		+							
Hayes	2085	ns	-	-	-	-	ns		-							
Hayes	2086	ns	-	+ 6	+ 6	c	-		+	-	-	-	-		-	
Hayes	2087	ns	-	-	-	-	ns		-							
Hayes	2089	ns	c	-	+ 6	-	ns		+	-	-	-	-		-	
Hayes	2090	ns	-	+ 6	+ 8	-	ns		+	-	-	-	-		-	
Hayes	2091	ns	-	-	-	-	ns		-							
Hayes	2092	ns	-	-	-	-	ns		-							
Hayes	2093	ns	-	-	-	-	ns		-							
Hayes	2094	ns	c	+ 11	c	-	-		+	-	-	-	-		-	Triplets (all -ve)
Hayes	2096	ns	-	+ 6	+ 6	-	ns		+							
Hayes	6002	-	+ 5	+ 6	+ 6	-	ns		+	-	-	-	+(7)		+	
Hayes	6003	-	-	-	c	ns	ns		-							
Hayes	6004	-	-	-	-	-	ns		-							
Hayes	6005	-	c	-	-	-	ns		-							
Hayes	6006	-	-	-	+ 9	-	-		+	-	-	-	-		-	Twins, both -ve)

Appendix 3. Culture results (ewes and foetuses)

Sheep details		Ewe results								Foetus results						
Source	Tag no.	Faeces 2000	Faeces	MLN	Ileum/ICV	Suprmammary lymph node	Milk/udder	Other	Ewe summary	MLN	Ileum/ICV	Spleen	Cotyledon	Other	Foetus summa	Comment
Hayes	6007	-	-	+ 5	+ 4	-	-		+	-	-	-	-		-	
Hayes	6008	-	-	+ 6	+ 5	-	-		+	-	-	-	-		-	
Hayes	6009	-	-	c	c	c	ns		-							
Hayes	6010	-	-	-	c	-	ns		-							
Hayes	6011	-	-	+ 7	-	-	-		+	-	-	-	-		-	
Hayes	6012	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	6013	-	-	-	-	-	ns		-							
Hayes	6014	-	-	-	-	-	ns		-							
Hayes	6015	-	c	-	-	-	-		-	-	-	-	-		-	
Hayes	6016	-	+ 4	+ 4	+ 2	-	-		+	-	-	-	-		-	
Hayes	6017	-	+ 7	+ 6	+ 5	-	-		+	-	-	-	-		-	
Hayes	6018	-	c	-	-	ns	ns		-							
Hayes	6019	-	-	-	-	-	ns		-							
Hayes	6020	-	-	+ 7	+ 6	-	-		+	-	-	-	-		-	
Hayes	6021	-	-	+ 6	+ 6	-	-		+	-	-	-	-		-	
Hayes	6022	+ 7	+ 5	+ 4	+ 2	-	ns		+	-	-	-	+ (6)		+	Twins (one +ve)
Hayes	6023	-	c	+ 12	-	-	-		+	-	-	-	-		-	Twins, both -ve)
Hayes	6024	-	-	+ 7	+ 6	-	c		+	-	c	-	-		-	
Hayes	6025	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	6026	-	c	-	-	-	ns		-							
Hayes	6029	-	c	-	-	-	ns		-	-	-	-	-		-	
Hayes	6030	-	-	+ 7	c	-	-		+	-	-	-	-		-	
Hayes	6031	-	-	+ 10	-	-	-		+	-	-	-	-		-	
Hayes	6032	-	-	-	-	-	ns		-							
Hayes	6033	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	6034	-	-	-	-	-	ns		-							
Hayes	6035	-	c	+ 6	c	-	-		+	-	-	-	-		-	
Hayes	6036	-	+ 6	+ 6	+ 4	-	-		+	-	-	-	-		-	
Hayes	6037	-	-	-	-	-	ns		-							
Hayes	6038	-	-	-	-	-	-		-	-	-	-	-		-	
Hayes	6040	-	-	-	-	-	ns		-							

Appendix 3. Culture results (ewes and foetuses)

Sheep details		Ewe results								Foetus results						
Source	Tag no.	Faeces 2000	Faeces	MLN	Ileum/ICV	Suprmammary lymph node	Milk/udder	Other	Ewe summary	MLN	Ileum/ICV	Spleen	Cotyledon	Other	Foetus summa	Comment
Hayes	6041	-	-	-	-	-	ns		-							
Hayes	6044	+ 7	-	-	-	-	ns		+							
Hayes	6046	-	-	-	-	-	ns		-							
Hayes	6047	-	-	+ 6	-	-	-		+	-	-	-	-		-	
Hayes	6048	-	-	-	-	c	-		-							
Hayes	6049	-	-	-	c	-	ns		-							
Hayes	6050	-	-	+ 7	+ 8	-	ns		+	-	-	-	-		-	
Hayes	6051	-	-	+ 7	-	-	-		+	-	-	-	-		-	
Hayes	6052	-	-	-	-	-	ns		-							
Hayes	6054	-	-	-	-	-	ns		-							
Hayes	6055	-	-	-	-	-	ns		-							
Hayes	6056	-	-	-	c	-	ns		-							
Hayes	6057	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	6058	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	6059	-	-	-	-	-	ns		-							
Hayes	6060	-	-	-	c	-	ns		-							
Hayes	6061	-	-	+ 6	+ 4	-	-		+	-	-	-	-		-	Twins (both -ve)
Hayes	6063	-	-	-	-	-	ns		-							
Hayes	6064	-	-	+ 9	+ 7	-	-		+	-	-	-	-		-	
Hayes	6065	-	-	-	-	-	ns		-							
Hayes	6066	-	-	c	-	c	ns		-							
Hayes	6067	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	6068	-	-	-	c	-	ns		-							
Hayes	6069	-	-	+ 11	c	-	-		+	-	-	-	-		-	
Hayes	6070	-	-	c	c	+ 6	-		+	-	-	-	-		-	
Hayes	6071	-	c	-	-	-	ns		-							
Hayes	6072	-	-	-	c	-	ns		-							
Hayes	6074	+ 11	+ 7	+ 5	+ 7	-	-		+	-	c	-	-		-	
Hayes	6075	-	-	-	c	-	ns		-							
Hayes	6077	-	-	-	-	-	ns		-							
Hayes	6078	-	-	-	-	-	ns		-							

Appendix 3. Culture results (ewes and foetuses)

Sheep details		Ewe results								Foetus results						
Source	Tag no.	Faeces 2000	Faeces	MLN	Ileum/ICV	Suprmammary lymph node	Milk/udder	Other	Ewe summary	MLN	Ileum/ICV	Spleen	Cotyledon	Other	Foetus summa	Comment
Hayes	6079	-	-	-	-	c	ns		-	-	-	-	-		-	
Hayes	6080	-	-	-	-	-	ns		-							
Hayes	6081	-	-	-	-	-	ns		-							
Hayes	6082	-	-	-	-	-	ns		-							
Hayes	6083	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	6084	-	-	-	-	-	ns		-							
Hayes	6085	-	-	-	-	-	ns		-							
Hayes	6086	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	6087	-	-	+ 8	-	-	-		+	-	-	-	-		-	Twins (both -ve)
Hayes	6088	-	-	+ 11	-	-	-		+	-	-	-	-		-	
Hayes	6089	-	+ 3	+ 5	+ 4	-	-		+	-	-	-	-		-	Twins (both -ve)
Hayes	6090	-	c	-	-	-	ns		-	-	-	-	-		-	
Hayes	6091	-	-	-	-	-	-		-							
Hayes	6092	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	6093	-	-	-	-	ns	ns		-							
Hayes	6094	-	-	+ 7	+ 7	-	-		+	-	-	-	-		-	
Hayes	6096	-	-	+ 5	+ 5	-	ns		+	-	-	-	-		-	
Hayes	6097	-	-	-	-	-	ns		-	-	-	-	-		-	
Hayes	6098	+ 11	+ 6	+ 6	+ 3	c	-		+	-	-	-	-		-	
Hayes	6099	-	-	-	-	-	ns		-							
Hayes	6100	-	-	-	+ 9	-	-		+	-	-	-	-		-	
S. Uni	P1	ns	+ 3	+ 4	+ 1	ns	+	uterineLN	+	-	-	-	+ (6)		+	
S. Uni	P	ns	-	-	-	ns	-	-	-	-	-	-	-		-	
S. Uni	O	ns	-	-	-	ns	-	uterus	+	-	-	-	+ (6)		+	
S. Uni	NT	ns	-	-	-	ns	-	-	-	-	-	-	-		-	
S. Uni	B1	ns	ns	+	+	ns	+	uterus	+	-	-	+	+	liver, umbilicus	+	
S. Uni	B	ns	ns	+	+	ns	-	uterus	+	-	-	+	+	liver	+	

Indicates positive culture

Numbers after positive results indicate weeks taken for Bactec growth index to reach 999

Appendix 4. Pathological findings (ewes)

Sheep	Gross lesions				Microscopic lesions										
					Ileum			Mesenteric lymph nodes				Final classification			
Tag no.	Ileal thickening	Lymphangitis	Enlarged MLNs	Summary	No. sections	Lesion severity (Marshall)	AFB (1-4, Marshall)	No. sections	No. nodes affected	Lesion severity (Marshall)	AFB (1-4, Marshall)	Score (Perez)	AFB	Description
1088	-	-	-	-	4	3	1	4	0	0		1	+	Focal lesions, PP only
1701	-	-	-	-	4	0		3	0	0		-	-	nsf
1702	+/-	+/-	-	-	3	0		2	0	0		-	-	nsf
1703	-	-	-	-	4	0		3	0	0		-	-	nsf
1705	-	-	-	-	3	2	0	3	0	0		1	-	Focal lesions, PP only
1706	-	-	-	-	4	0		2	0	0		-	-	nsf
1707	-	-	-	-	4	0		4	0	0		-	-	nsf
1708	-	-	-	-	4	2	1	3	0	0		2	+	Focal lesions, PP & mucosa
1709	-	-	-	-	4	0		5	0	0		-	-	nsf
1710	-	-	-	-	4	0		4	3	2	0	2n	-	Focal lesions, node only
1711	-	-	-	-	4	3	0	3	2	1	1	2	+	Focal lesions, PP & mucosa
1712	-	-	-	-	4	3	0	2	1	1	0	2	-	Focal lesions, PP & mucosa
1713	-	-	-	-	3	0		3	0	0		-	-	nsf
1714	-	-	-	-	3	0		3	0	0		-	-	nsf
1715	+	+	-	+	3	7	4	4	4	2	1	3b	+	Severe diffuse multibacillary
1716	-	-	-	-	3	1	0	3	0	0		-	-	Equivocal, pigmented macrophage clumps
1717	+	-	-	+	2	4	1	4	3	2	2	3a	+	Multifocal lesions
1718	-	-	-	-	4	0		3	0	0		-	-	nsf
1719	-	-	-	-	4	0		3	0	0		-	-	nsf
1720	-	-	-	-	3	0		4	0	0		-	-	nsf
1721	+	-	-	+	3	1	0	3	0	0		-	-	Equivocal, mineralising granuloma
1723	-	-	-	-	4	2	1	4	0	0		2	+	Focal lesions, PP & mucosa
1724	-	-	-	-	4	0		4	0	0		-	-	nsf
1725	-	-	-	-	4	2	0	3	0	0		1	-	Focal lesions, PP only
2062	-	-	-	-	5	0		3	0	0		-	-	nsf
2063	-	-	-	-	3	0		3	0	0		-	-	nsf
2064	-	-	-	-	4	0		3	0	0		-	-	nsf

Appendix 4. Pathological findings (ewes)

Sheep	Gross lesions				Microscopic lesions										
					Ileum			Mesenteric lymph nodes				Final classification			
Tag no.	Ileal thickening	Lymphangitis	Enlarged MLNs	Summary	No. sections	Lesion severity (Marshall)	AFB (1-4, Marshall)	No. sections	No. nodes affected	Lesion severity (Marshall)	AFB (1-4, Marshall)	Score (Perez)	AFB	Description
2065	-	-	+/-	-	4	0		3	0	0		-	-	nsf
2066	-	-	-	-	4	0		3	0	0		-	-	nsf
2067	-	-	-	-	3	0		3	0	0		-	-	nsf
2068	-	-	-	-	4	0		3	0	0		-	-	nsf
2069	-	-	-	-	3	0		3	0	0		-	-	nsf
2070	-	-	-	-	4	1	0	3	0	0		-	-	Equivocal, pigmented macrophage clumps
2071	-	-	-	-	4	1		3	0	0		-	-	Equivocal, fibrosed granuloma
2072	-	-	-	-	4	0		3	0	0		-	-	nsf
2073	-	-	-	-	4	0		4	0	0		-	-	nsf
2074	-	-	-	-	4	0		4	0	0		-	-	nsf
2075	-	-	-	-	4	0		3	0	0		-	-	nsf
2076				-	4	0		3	0	0		-	-	nsf
2077	+	-	-	+	3	0		4	0	0		-	-	nsf
2078	-	-	-	-	4	0		4	0	0		-	-	nsf
2079	-	-	-	-	4	0		3	0	0		-	-	nsf
2080	-	-	-	-	3	0		3	0	0		-	-	nsf
2081	-	-	+/-	-	4	0		3	0	0		-	-	nsf
2082	-	-	-	-	3	0		4	0	0		-	-	nsf
2083	-	-	-	-	3	0		4	0	0		-	-	nsf
2084	-	-	-	-	4	0		3	0	0		-	-	nsf
2085	-	-	-	-	4	0		4	0	0		-	-	nsf
2086	+/-	-	-	-	4	4	1	3	2	2	0	3a	+	Multifocal lesions
2087	-	-	-	-	5	0		3	0	0		-	-	nsf
2089	-	-	-	-	4	2	1	3	0	0		1	+	Focal lesions, PP only
2090	+	+/-	+/-	+	3	5	0	2	2	1	0	3c	-	Paucibacillary, severe diffuse
2091	-	-	-	-	4	0		4	0	0		-	-	nsf
2092	-	-	-	-	4	0		3	0	0		-	-	nsf

Appendix 4. Pathological findings (ewes)

Sheep	Gross lesions				Microscopic lesions										
					Ileum			Mesenteric lymph nodes				Final classification			
Tag no.	Ileal thickening	Lymphangitis	Enlarged MLNs	Summary	No. sections	Lesion severity (Marshall)	AFB (1-4, Marshall)	No. sections	No. nodes affected	Lesion severity (Marshall)	AFB (1-4, Marshall)	Score (Perez)	AFB	Description
2093	-	-	+/-	-	4	0		2	0	0		-	-	nsf
2094	-	-	-	-	4	0		3	0	0		-	-	nsf
2096	-	-	-	-	3	4	1	4	3	2	1	3a	+	Multifocal lesions
6002	+	+	+	+	3	5	1	3	3	3	1	3c	+	Paucibacillary, severe diffuse
6003	-	-	-	-	4	0		3	0	0		-	-	nsf
6004	-	+/-	+/-	-	4	0		3	0	0		-	-	nsf
6005	-	-	-	-	4	0		4	0	0		-	-	nsf
6006	-	-	-	-	4	0		3	0	0		-	-	nsf
6007	-	-	-	-	5	5	0	3	1	2	0	3c	-	Paucibacillary, severe diffuse
6008	-	-	-	-	4	1	0	4	0	0		-	-	Equivocal, focal pyogranuloma
6009	-	-	-	-	4	0		3	0	0		-	-	nsf
6010	-	-	-	-	3	1	0	2	0	0		-	-	Equivocal, pigmented macrophage clumps
6011	-	-	-	-	4	4	1	3	3	2	1	3c	+	Paucibacillary, severe diffuse
6012	-	-	-	-	4	0		3	0	0		-	-	nsf
6013	-	-	-	-	4	0		3	0	0		-	-	nsf
6014	-	-	-	-	3	0		4	0	0		-	-	nsf
6015	+	-	-	+	3	0		3	0	0		-	-	nsf
6016	+ (jeju	+	-	+	3	6	4	3	3	2	3	3b	+	Severe diffuse multibacillary
6017	+/-	-	+/-	-	4	4	0	3	3	2	0	3a	-	Multifocal lesions
6018	-	-	-	-	3	0		3	0	0		-	-	nsf
6019	-	-	-	-	4	0		3	0	0		-	-	nsf
6020	-	-	+/-	-	4	2	1	4	0	0		2	+	Focal lesions, PP & mucosa
6021	+	-	-	+	4	4	2	4	2	2	1	3a	+	Multifocal lesions
6022	+	+	+	+	3	7	4	3	3	2	0	3b	+	Severe diffuse multibacillary
6023	-	-	-	-	4	0		4	0	0		-	-	nsf
6024	+	+/-	+	+	4	6	4	4	4	2	2	3b	+	Severe diffuse multibacillary
6025	-	-	-	-	3	0		3	0	0		-	-	nsf

Appendix 4. Pathological findings (ewes)

Sheep	Gross lesions				Microscopic lesions										
					Ileum			Mesenteric lymph nodes				Final classification			
Tag no.	Ileal thickening	Lymphangitis	Enlarged MLNs	Summary	No. sections	Lesion severity (Marshall)	AFB (1-4, Marshall)	No. sections	No. nodes affected	Lesion severity (Marshall)	AFB (1-4, Marshall)	Score (Perez)	AFB	Description
6026	-	-	+	+	4	0		3	0	0		-	-	nsf
6029	-	-	-	-	4	0		3	0	0		-	-	nsf
6030	-	-	-	-	4	0		3	0	0		-	-	nsf
6031	-	-	+	+	4	0		3	0	0		-	-	nsf
6032	-	-	-	-	4	0		4	0	0		-	-	nsf
6033	+	-	+	+	4	2	1	4	1	1	0	1	+	Focal lesions, PP only
6034	-	-	-	-	4	0		4	0	0		-	-	nsf
6035	-	-	-	-	4	0		4	0	0		-	-	nsf
6036	+	-	-	+	3	4	1	4	3	1	0	3a	+	Multifocal lesions
6037	-	-	-	-	3	0		3	0	0		-	-	nsf
6038	-	-	-	-	4	2	1	2	0	0		1	+	Focal lesions, PP only
6040	-	-	-	-	4	0		3	0	0		-	-	nsf
6041	-	-	-	-	4	0		4	0	0		-	-	nsf
6044	-	-	-	-	4	0		4	0	0		-	-	nsf
6046	-	+	-	-	4	0		3	0	0		-	-	nsf
6047	-	-	+/-	-	4	0		3	0	0		-	-	nsf
6048	-	-	-	-	4	0		4	0	0		-	-	nsf
6049	-	-	-	-	4	0		2	0	0		-	-	nsf
6050	-	-	-	-	3	0		3	0	0		-	-	nsf
6051	-	-	-	-	4	0		3	0	0		-	-	nsf
6052	-	-	-	-	4	0		4	0	0		-	-	nsf
6054	-	-	-	-	4	0		3	0	0		-	-	nsf
6055	-	-	-	-	4	0		3	0	0		-	-	nsf
6056	-	-	-	-	3	0		3	0	0		-	-	nsf
6057	-	-	-	-	4	0		3	0	0		-	-	nsf
6058	-	-	-	-	4	0		3	0	0		-	-	nsf
6059	-	-	-	-	4	0		3	0	0		-	-	nsf

Appendix 4. Pathological findings (ewes)

Sheep	Gross lesions				Microscopic lesions										
					Ileum			Mesenteric lymph nodes				Final classification			
Tag no.	Ileal thickening	Lymphangitis	Enlarged MLNs	Summary	No. sections	Lesion severity (Marshall)	AFB (1-4, Marshall)	No. sections	No. nodes affected	Lesion severity (Marshall)	AFB (1-4, Marshall)	Score (Perez)	AFB	Description
6060	-	-	-	-	3	0		4	0	0		-	-	nsf
6061	+/-	+	+	+	4	6	1	3	3	3	0	3c	+	Paucibacillary, severe diffuse
6063	-	-	-	-	4	0		4	0	0		-	-	nsf
6064	-	-	-	-	3	2	0	3	1	1	0	1	-	Focal lesions, PP only
6065	-	-	-	-	4	0		4	0	0		-	-	nsf
6066	+/-	-	-	-	4	0		4	0	0		-	-	nsf
6067	-	-	-	-	5	0		3	0	0		-	-	nsf
6068	-	-	-	-	5	0		4	0	0		-	-	nsf
6069	-	-	-	-	4	0		3	2	1	1	2n	+	Focal lesions, node only
6070	-	-	-	-	4	0		3	0	0		-	-	nsf
6071	-	-	-	-	3	0		4	0	0		-	-	nsf
6072	-	-	-	-	4	0		3	0	0		-	-	nsf
6074	-	-	-	-	4	2	1	3	2	2	1	2	+	Focal lesions, PP & mucosa
6075	-	-	-	-	3	0		3	0	0		-	-	nsf
6077	-	-	-	-	4	0		3	0	0		-	-	nsf
6078	-	-	-	-	4	0		3	0	0		-	-	nsf
6079	-	-	-	-	3	0		3	0	0		-	-	nsf
6080	-	-	-	-	4	0		3	0	0		-	-	nsf
6081	-	-	-	-	3	0		3	0	0		-	-	nsf
6082	+/-	-	-	-	4	0		4	0	0		-	-	nsf
6083	-	-	-	-	4	0		3	0	0		-	-	nsf
6084	-	-	-	-	4	0		4	0	0		-	-	nsf
6085	-	-	-	-	3	1	0	4	0	0		-	-	Equivocal, pigmented macrophage clumps
6086	-	-	-	-	4	2	1	3	0	0		1	+	Focal lesions, PP only
6087	-	-	-	-	4	0		3	0	0		-	-	nsf
6088	-	-	-	-	4	1	0	5	0	0		-	-	Equivocal, focal pyogranuloma
6089	+	+	+	+	4	5	4	3	3	2	1	3b	+	Severe diffuse multibacillary

Appendix 4. Pathological findings (ewes)

Sheep	Gross lesions				Microscopic lesions										
					Ileum			Mesenteric lymph nodes				Final classification			
Tag no.	Ileal thickening	Lymphangitis	Enlarged MLNs	Summary	No. sections	Lesion severity (Marshall)	AFB (1-4, Marshall)	No. sections	No. nodes affected	Lesion severity (Marshall)	AFB (1-4, Marshall)	Score (Perez)	AFB	Description
6090	-	-	-	-	4	1	0	3	0	0		-	-	Equivocal, pigmented macrophage clumps
6091	-	-	-	-	4	0		3	0	0		-	-	nsf
6092	-	-	-	-	4	0		3	0	0		-	-	nsf
6093	-	-	-	-	5	0		4	0	0		-	-	nsf
6094	-	-	-	-	4	0		3	0	0		-	-	nsf
6096	-	-	-	-	4	4	0	3	2	1	0	3c	+	Paucibacillary, severe diffuse
6097	-	-	-	-	3	0		4	2	1	1	2n	+	Focal lesions, node only
6098	-	-	+/-	-	4	4	3	3	3	2	0	3a	+	Multifocal lesions
6099	-	-	-	-	4	0		4	0	0		-	-	nsf
6100	-	-	-	-	4	2	0	4	0	0		1	-	Focal lesions, PP only

Appendix 5. Overview of summary results (ewes and foetuses)

		Antemortem tests		Necropsy samples (ewe)				Ewe status		Necropsy samples (foetus)				
				Culture		Pathology				Culture				
Source	Tag no.	Routine tests[1]	Exhaustive tests[2]	All tissues	Milk and/or udder	Grade	AFB	Infected	Clinical OJD confirmed	Cotyledon only	Other tissues	Number of foetuses	Crown-rump lengths	Comments
Hayes	1088	-	-	-	ns	1	+	+		-	-	2	465	1/2 taken
Hayes	1701	-	-	-	ns	-	-	-		ns	ns	2	380	1/2 taken
Hayes	1702	-	-	-	ns	-	-	-		ns	ns	2	420, 440	
Hayes	1703	-	-	-	ns	-	-	-		-	-	2	485	1/2 taken
Hayes	1705	-	+	-	ns	1	-	+		-	-	2	440	1/2 taken, no milk
Hayes	1706	-	+	-	ns	-	-	-		ns	ns	2	415	1/2 taken
Hayes	1707	-	-	-	ns	-	-	-		ns	ns	2	480	1/2 taken
Hayes	1708	-	-	+	ns	2	+	+		-	-	2	410	1/2 taken, no milk
Hayes	1709	-	-	-	ns	-	-	-		ns	ns	2	415	1/2 taken
Hayes	1710	-	-	-	ns	2n	-	+		-	-	2	420	1/2 taken
Hayes	1711	-	-	+	-	2	+	+		-	-	2	420	1/2 taken
Hayes	1712	+	+	+	-	2	-	+		-	-	1	500	
Hayes	1713	-		-	ns	-	-	-		ns	ns	1	465	
Hayes	1714	-		-	ns	-	-	-		ns	ns	2	420	1/2 taken
Hayes	1715	+	+	+	-	3b	+	+		-	-	2	420	1/2 taken
Hayes	1716	-	+	-	ns	-	-	-		-	-	2	460	
Hayes	1717	-	+	+	-	3a	+	+		-	-	1	455	
Hayes	1718	-		-	ns	-	-	-		ns	ns	1	470	
Hayes	1719	-		-	ns	-	-	-		-	-	2	460	1/2 taken, little milk
Hayes	1720	-	+	-	ns	-	-	-		ns	ns	1	465	
Hayes	1721	-		+	ns	-	-	+		-	-	2	370	1/2 taken, no milk
Hayes	1723	-	+	+	-	2	+	+		-	-	1	530	
Hayes	1724	-	+	-	ns	-	-	-		ns	ns	2	435	1/2 taken, no milk
Hayes	1725	-	+	-	ns	1	-	+		-	-	2	330	1/2 taken
Hayes	2062	-	-	-	ns	-	-	-		ns	ns	1	465	
Hayes	2063	-	-	-	ns	-	-	-		ns	ns	2	480	1/2 taken, little milk
Hayes	2064	+	+	-	ns	-	-	-		ns	ns	2	430	1/2 taken

Appendix 5. Overview of summary results (ewes and foetuses)

		Antemortem tests		Necropsy samples (ewe)				Ewe status		Necropsy samples (foetus)				
				Culture		Pathology				Culture				
Source	Tag no.	Routine tests[1]	Exhaustive tests[2]	All tissues	Milk and/or udder	Grade	AFB	Infected	Clinical OJD confirmed	Cotyledon only	Other tissues	Number of foetuses	Crown-rump lengths	Comments
Hayes	2065	-	-	+[3]	-	-	-	+		-	-	2	420	
Hayes	2066	-	-	-	ns	-	-	-		ns	ns	2	435	1/2 taken
Hayes	2067	-	-	-	ns	-	-	-				0		empty
Hayes	2068	-	-	-	ns	-	-	-		ns	ns	2	395	1/2 taken
Hayes	2069	-	+	-	ns	-	-	-		ns	ns	2	440	1/2 taken
Hayes	2070	-	-	-	ns	-	-	-		-	-	2	410, 400	
Hayes	2071	-	-	+	-	-	-	+		-	-	1	430	
Hayes	2072	-	-	+	-	-	-	+		-	-	2	490	1/2 taken
Hayes	2073	-	-	-	ns	-	-	-				0		empty
Hayes	2074	-	-	-	ns	-	-	-		ns	ns	1	485	
Hayes	2075	-	-	-	-	-	-	-		-	-	2	455	1/2 taken
Hayes	2076	-	-	-	ns	-	-	-		ns	ns	1	410	
Hayes	2077	-	-	-	ns	-	-	-		ns	ns	1	565	
Hayes	2078	-	-	-	ns	-	-	-		ns	ns	2	280	1/2 taken
Hayes	2079	-	-	-	ns	-	-	-		ns	ns	2	400	1/2 taken, abundant colostrum
Hayes	2080	-	+	-	ns	-	-	-		ns	ns	2	480	1/2 taken
Hayes	2081	+	+	-	ns	-	-	-		ns	ns	2	415	1/2 taken
Hayes	2082	-	-	-	ns	-	-	-		ns	ns	1	415	
Hayes	2083	-	-	-	ns	-	-	-		ns	ns	2	480	1/2 taken
Hayes	2084	-	-	+[3]	ns	-	-	+		ns	ns	2	335	1/2 taken
Hayes	2085	-	-	-	ns	-	-	-		ns	ns	1	445	
Hayes	2086	-	-	+	-	3a	+	+		-	-	2	255	1/2 taken
Hayes	2087	-	-	-	ns	-	-	-		ns	ns	2	415	1/2 taken
Hayes	2089	-	-	+	ns	1	+	+		-	-	1	355	no milk
Hayes	2090	+	+	+	ns	3c	-	+		-	-	2	375	1/2 taken, no milk, calcified lump in ICV
Hayes	2091	-	-	-	ns	-	-	-		ns	ns	2	410	1/2 taken

Appendix 5. Overview of summary results (ewes and foetuses)

		Antemortem tests		Necropsy samples (ewe)				Ewe status		Necropsy samples (foetus)				
				Culture		Pathology				Culture				
Source	Tag no.	Routine tests[1]	Exhaustive tests[2]	All tissues	Milk and/or udder	Grade	AFB	Infected	Clinical OJD confirmed	Cotyledon only	Other tissues	Number of foetuses	Crown-rump lengths	Comments
Hayes	2092	-	-	-	ns	-	-	-		ns	ns	2	420	1/2 taken
Hayes	2093	-	-	-	ns	-	-	-		ns	ns	1	480	
Hayes	2094	+	+	+	-	-	-	+	+	-	-	3	430, 440, 445	
Hayes	2096	-	-	+	ns	3a	+	+				0		empty
Hayes	6002	+	+	+	ns	3c	+	+	+	+	-	1	445	no milk
Hayes	6003	-	-	-	ns	-	-	-				0		empty
Hayes	6004	-	+	-	ns	-	-	-		ns	ns	2	430, 400	
Hayes	6005	-	-	-	ns	-	-	-		ns	ns	1	555	
Hayes	6006	-	+	+	-	-	-	+		-	-	2	435, 445	
Hayes	6007	-	+	+	-	3c	-	+		-	-	2	420	1/2 taken
Hayes	6008	-	+	+	-	-	-	+		-	-	1	480	
Hayes	6009	-	-	-	ns	-	-	-		ns	ns	1	445	
Hayes	6010	+	+	-	ns	-	-	-		ns	ns	3	440, 380, 390	
Hayes	6011	-	-	+	-	3c	+	+		-	-	3	395	1/3 taken
Hayes	6012	-	-	-	ns	-	-	-		-	-	2	420	1/2 taken
Hayes	6013	-	+	-	ns	-	-	-				0		empty
Hayes	6014	-	+	-	ns	-	-	-		ns	ns	2	440	1/2 taken
Hayes	6015	-	-	-	-	-	-	-		-	-	2	425	1/2 taken
Hayes	6016	+	+	+	-	3b	+	+		-	-	2	415	1/2 taken
Hayes	6017	+	+	+	-	3a	-	+		-	-	1	490	
Hayes	6018	-	-	-	ns	-	-	-				0		empty
Hayes	6019	-	-	-	ns	-	-	-		ns	ns	1	415	no milk
Hayes	6020	+	+	+	-	2	+	+		-	-	1	430	
Hayes	6021	-	-	+	-	3a	+	+		-	-	1	460	
Hayes	6022	+	+	+	ns	3b	+	+	+	+	-	2	350, 340	no milk
Hayes	6023	-	+	+	-	-	-	+		-	-	2	445, 460	

Appendix 5. Overview of summary results (ewes and foetuses)

		Antemortem tests		Necropsy samples (ewe)				Ewe status		Necropsy samples (foetus)				
				Culture		Pathology				Culture				
Source	Tag no.	Routine tests[1]	Exhaustive tests[2]	All tissues	Milk and/or udder	Grade	AFB	Infected	Clinical OJD confirmed	Cotyledon only	Other tissues	Number of foetuses	Crown-rump lengths	Comments
Hayes	6024	+	+	+	c	3b	+	+		-	-	1	490	
Hayes	6025	-	-	-	ns	-	-	-		-	-	1	410	
Hayes	6026	-	-	-	ns	-	-	-				0		empty
Hayes	6029	-	-	-	ns	-	-	-		-	-	2	435	1/2 taken
Hayes	6030	-	-	+	-	-	-	+		-	-	1	390	
Hayes	6031	-	-	+	-	-	-	+		-	-	1	460	
Hayes	6032	-	+	-	ns	-	-	-		ns	ns	1	475	
Hayes	6033	-	-	-	ns	1	+	+		-	-	1	495	
Hayes	6034	-	-	-	ns	-	-	-		ns	ns	2	495	1/2 taken
Hayes	6035	-	-	+	-	-	-	+		-	-	2	460	1/2 taken
Hayes	6036	+	+	+	-	3a	+	+		-	-	1	540	
Hayes	6037	-	+	-	ns	-	-	-		ns	ns	2	450, 440	
Hayes	6038	-	-	-	-	1	+	+		-	-	2	470	1/2 taken
Hayes	6040	-	-	-	ns	-	-	-		ns	ns	2	465	1/2 taken
Hayes	6041	-	-	-	ns	-	-	-		ns	ns	2	480	1/2 taken
Hayes	6044	-	+	-	ns	-	-	+		ns	ns	2	435	1/2 taken
Hayes	6046	-	-	-	ns	-	-	-		ns	ns	2	425, 410	
Hayes	6047	-	-	+	-	-	-	+		-	-	2	425	1/2 taken, little milk
Hayes	6048	-	+	-	-	-	-	-		ns	ns	2	365	1/2 taken
Hayes	6049	-	-	-	ns	-	-	-		ns	ns	1	400	
Hayes	6050	-	+	+	ns	-	-	+		-	-	1	425	little milk
Hayes	6051	-	-	+	-	-	-	+		-	-	2	385	1/2 taken
Hayes	6052	-	-	-	ns	-	-	-		ns	ns	1	445	
Hayes	6054	-	-	-	ns	-	-	-		ns	ns	1	490	
Hayes	6055	+	+	-	ns	-	-	-		ns	ns	2	430, 445	
Hayes	6056	-	-	-	ns	-	-	-		ns	ns	2	430	1/2 taken

Appendix 5. Overview of summary results (ewes and foetuses)

		Antemortem tests		Necropsy samples (ewe)				Ewe status		Necropsy samples (foetus)				
				Culture		Pathology				Culture				
Source	Tag no.	Routine tests[1]	Exhaustive tests[2]	All tissues	Milk and/or udder	Grade	AFB	Infected	Clinical OJD confirmed	Cotyledon only	Other tissues	Number of foetuses	Crown-rump lengths	Comments
Hayes	6057	-	-	-	ns	-	-	-		-	-	2	415	1/2 taken
Hayes	6058	-	-	-	ns	-	-	-		-	-	1	455	
Hayes	6059	-	+	-	ns	-	-	-		ns	ns	2	345	1/2 taken
Hayes	6060	-	-	-	ns	-	-	-				0		
Hayes	6061	+	+	+	-	3c	+	+		-	-	2	400, 390	
Hayes	6063	-	+	-	ns	-	-	-		ns	ns	2	295	1/2 taken
Hayes	6064	-	+	+	-	1	-	+		-	-	1	500	
Hayes	6065	-	-	-	ns	-	-	-		ns	ns	1	405	
Hayes	6066	-	+	-	ns	-	-	-		ns	ns	2	445	1/2 taken
Hayes	6067	-	-	-	ns	-	-	-		-	-	2	490	1/2 taken, little milk
Hayes	6068	-	-	-	ns	-	-	-		ns	ns	1	420	
Hayes	6069	-	-	+	-	2n	+	+		-	-	2	420	1/2 taken
Hayes	6070	-	-	+	-	-	-	+		-	-	2	335	1/2 taken
Hayes	6071	-	-	-	ns	-	-	-		ns	ns	1	450	
Hayes	6072	-	-	-	ns	-	-	-		ns	ns	2	440	1/2 taken
Hayes	6074	+	+	+	-	2	+	+		-	-	2	410	1/2 taken
Hayes	6075	-	-	-	ns	-	-	-		ns	ns	2	430, 435	
Hayes	6077	-	-	-	ns	-	-	-		ns	ns	1	515	
Hayes	6078	-	-	-	ns	-	-	-		ns	ns	2	420	1/2 taken
Hayes	6079	-	+	-	ns	-	-	-		-	-	1	380	
Hayes	6080	-	-	-	ns	-	-	-		ns	ns	2	390	1/2 taken
Hayes	6081	-	+	-	ns	-	-	-		ns	ns	3	415	1/3 taken, no milk
Hayes	6082	-	-	-	ns	-	-	-		ns	ns	1	360	little milk
Hayes	6083	-	-	-	ns	-	-	-		-	-	1	505	
Hayes	6084	-	-	-	ns	-	-	-		ns	ns	1	435	
Hayes	6085	-	+	-	ns	-	-	-		ns	ns	2	450	1/2 taken

Appendix 5. Overview of summary results (ewes and foetuses)

		Antemortem tests		Necropsy samples (ewe)				Ewe status		Necropsy samples (foetus)				
				Culture		Pathology				Culture				
Source	Tag no.	Routine tests[1]	Exhaustive tests[2]	All tissues	Milk and/or udder	Grade	AFB	Infected	Clinical OJD confirmed	Cotyledon only	Other tissues	Number of foetuses	Crown-rump lengths	Comments
Hayes	6086	-	+	-	ns	1	+	+		-	-	2	365	1/2 taken, abundant colostrum
Hayes	6087	-	-	+	-	-	-	+		-	-	2	420, 420	
Hayes	6088	-	-	+	-	-	-	+		-	-	2	405	1/2 taken
Hayes	6089	+	+	+	-	3b	+	+		-	-	2	440, 440	
Hayes	6090	-	-	-	ns	-	-	-		-	-	1	515	
Hayes	6091	-	+	-	-	-	-	-		ns	ns	1	440	
Hayes	6092	-	-	-	ns	-	-	-		-	-	1	480	
Hayes	6093	-	+	-	ns	-	-	-				0		empty
Hayes	6094	-	-	+	-	-	-	+		-	-	2	435	1/2 taken
Hayes	6096	-	+	+	ns	3c	-	+		-	-	1	300	
Hayes	6097	-	+	-	ns	2n	+	+		-	-	1	440	no milk
Hayes	6098	+	+	+	-	3a	+	+		-	-	2	435	1/2 taken, little milk
Hayes	6099	-	-	-	ns	-	-	-		ns	ns	2	470	1/2 taken
Hayes	6100	+	+	+	-	1	-	+		-	-	2	420	1/2 taken
S. Uni	P1	+	+	+	+			+	+	+	-			
S. Uni	P	+	+	-	-			-		-	-			
S. Uni	O	+	+	+[4]	-			+	+[4]	+	-			
S. Uni	NT	+	+	-	-			-		-	-			
S. Uni	B1	+	+	+	+			+	+	+	+			
S. Uni	B	+	+	+	-			+	+	+	+			

ns Not sampled/tested

[1] Includes clinical status, faecal culture and gel test results from 2001 only

[2] Includes all 2001 and 2000 results

[3] Positive only in supramammary lymph node

[4] Positvie only in uterus, unable to confirm that clinical signs were definitely due to OJD, so classified as subclinical