

On farm

Individual animal tests for Ovine Johne's disease

Project number OJD.020

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ABSTRACT

Routine diagnostic tests for ovine paratuberculosis have poor sensitivity in the early stages of the disease, and transmission often occurs before detection. Currently there are no tests to accurately confirm early infection in individual sheep. Such tests are required to provide trading opportunities for producers who may have valuable stock at low risk of infection. Surgical biopsy is one means of disease detection using relatively sensitive laboratory procedures, but was unproven. 77 sheep grazing on a heavily infected farm were examined at 12, 18 and 24 months of age by histopathology and culture of biopsied ileum and mesenteric lymph nodes. Results from biopsy were compared to those from routine tests (ELISA, AGID, IFN- γ , skin testing, faecal culture and direct PCR) applied at six-monthly intervals, and to necropsy findings at three years of age. A total of 170 biopsies were performed without serious complications, and the samples collected were adequate for culture and histological assessment of paratuberculosis. Overall, 36% of sheep were shown to be uninfected at 3 years of age. Of these, 16 were uninfected at all sampling times and 11 sheep had recovered. (ie. They had been infected at an earlier sampling.) The remaining 64% of sheep were classified at necropsy as infected. These included 36 sheep with subclinical OJD (6 of these had severe diffuse lesions, and 30 had milder lesions), and 11 sheep which had died of OJD. Biopsy was consistently the most sensitive non-lethal technique for identification of infected sheep, although even at 36 months it detected only 2/3 of infected sheep. It may be useful as an additional tool in the management for individual valuable sheep from infected stud flocks. Because some sheep do recover from early infection with *M. a. paratuberculosis*, early testing at 12 months of age, even with a sensitive test such as culture of biopsied tissue, was not be predictive of later infection status. By 18 to 30 months old, positive test results for CMI, humoral immunity or faecal excretion, were often highly associated with later death from OJD, although not often with infection status at 3 years. The lack of statistical association with final infection status reflects the low sensitivity of these tests at this early stage of the disease.

EXECUTIVE SUMMARY

Routine diagnostic tests for ovine Johne's disease (OJD) have poor sensitivity in the early stages of the disease, and transmission often occurs before detection. Most of the tests used in the OJD program are flock tests. There is a need for an accurate test or testing strategy for individual animals, particularly where it is desired to show with confidence that a given individual is not infected. Such tests are required to provide trading opportunities for producers who may have valuable stock at low risk of infection. In order to exploit existing tests and develop new strategies for their use in individual animals, greater understanding of the progression of the infection in individuals was required as well as better knowledge about the behaviour of tests at different stages of the disease.

The current investigation was a longitudinal study in which the results of tests, in particular intestinal biopsies, at each time point were used to define the progression of disease in each animal and to determine the correlation of test results with the infection status at 3 years of age.

77 fine-wool Merino sheep were grazed on a severely OJD affected property near Goulburn, NSW. The sheep were run under normal farm conditions. Each sheep was examined using a range of tests at regular intervals. In addition, the sheep were examined closely at least weekly by the farmer, and weights and condition scores were recorded at the time of each biopsy examination.

The tests applied at 9,12,18,24,30 and 36 months of age were as follows. Tests for humoral antibody included the Elisa (Parachek, CSL) and agar gel immunodiffusion test (AGID). The IFN- γ test (Bovigam, CSL) and skin testing for delayed type hypersensitivity (DTH) were used to assess cell mediated immunity. Faecal excretion of *M. a. paratuberculosis* was assessed by individual faecal culture (IFC) and direct PCR on faeces (D-PCR).

Biopsy specimens (1-2 g samples of terminal ileum and mesenteric lymph node) were collected from all sheep at 12 and 24 months of age, and from 20 selected sheep at 18 months. These were cultured for *M. a. paratuberculosis* and processed for histopathology. Biopsy surgery was performed by five different surgeons in a veterinary clinic under anaesthesia using sterile procedures. Prophylactic antibiotics and analgesics were used. The sheep tolerated handling and transport without obvious signs of distress. They remained quiet and easy to manage in the clinical environment. A total of 170 laparotomies were performed by five veterinarians without any serious complications. Mean surgical operating time was 46 minutes. The samples collected at biopsy were adequate for culture and histological assessment of paratuberculosis.

All surviving sheep were killed at 3 years of age using intravenous barbiturate. Throughout the trial, sheep with severe clinical signs, were similarly euthanased. Five gram samples of terminal ileum (3 sites), ICV, and MLN (3 sites) were collected into sterile containers for culture, and adjacent tissues were processed for histopathology. In addition, samples identical to those collected at biopsy were collected (mock-biopsies).

A sheep was classified as infected at a particular sampling time if it had direct evidence of *M. a. paratuberculosis* infection, viz. a positive faecal or tissue culture, positive D-PCR or histological lesions consistent with OJD. If all these tests were negative, the sheep was classified as uninfected at that sampling. Over the course of the trial, 11 sheep were euthanased with severe OJD. No other sheep displayed clinical signs. Three sheep died in the paddock so could not be assigned a final OJD status, and were excluded from statistical analysis. Thus 63 sheep were finally necropsied at 36 months, and a total of 74 sheep were available for detailed analysis of test performance.

27 sheep (36%) were found to be uninfected at 3 years of age. Of these, 16 were uninfected at all sampling times (5 had a positive result on at least one occasion in the Elisa, AGID, IFN- γ or skin test, and 11 had no positive immunological reactions). The remaining 11 uninfected sheep (4 with no immunological reactions) had been infected at an earlier sampling, and were considered to have recovered from infection. 47 sheep (64%) were classified as infected at necropsy. These included 36 sheep with subclinical OJD (6 of these had severe diffuse lesions, and 30 had milder lesions), and the 11 sheep which had died of OJD.

At 9 months, 10% of sheep were positive by IFC. Passive excretion may partially explain these results. Faecal culture status at 18, 24 and 30 months was highly predictive of a fatal outcome, but not statistically associated with final infection status. At the 36 month sampling, all IFC positive sheep had OJD lesions, and 5 of these had severe diffuse OJD lesions, a highly significant association. D-PCR results followed a very similar pattern to the IFC results, but D-PCR was less sensitive when small numbers of organisms were present.

All sheep were negative at 9 and 12 months in the AGID. Positive AGID tests at 24 and 30 months were significantly associated with later death from OJD but not with final infection status. 9 of the 11 sheep that died of OJD had a positive AGID test at necropsy. Positive ELISA results at 18, 24 and 30 months were associated with death from OJD, but not with final infection status. Of 8 sheep which were tested in the ELISA at necropsy, 7 were positive.

Positive IFN- γ results at 18 and 24 months were highly associated with later death from OJD, but not significantly associated with final infection status. Many sheep (48%) were IFN- γ positive at 30 months, and at this time there was a significant association with final infection status. At 36 months IFN- γ was not helpful to identify either infected sheep or those with severe lesions. Many sheep (32%) had positive skin tests at 24 months, and positive results were significantly associated with later death from OJD. Few sheep reacted in the later samplings, and there were no significant associations with final status.

Culture of tissues obtained by biopsy at 12 months detected many more infected sheep than any other antemortem test. There was no significant association between these results and final infection status at 3 years of age, but positive biopsy results at this time were significantly associated with later death from OJD. Histopathology at this age was not sensitive. At 24 months, biopsy detected about twice as many infected sheep as most other tests, and biopsy results were highly predictive of fatal outcome. Culture from biopsy samples at this time was significantly associated with final infection status and with later death from OJD.

For early infection, up to 12 months exposure, culture from mock-biopsy samples detected 45% of sheep found to be infected at necropsy at that time, and histopathology detected no additional infected sheep. These results were obtained from the tracer weaner studies conducted on the same farm. At 36 months, culture of mock-biopsy samples detected 53% of sheep confirmed to be infected at necropsy, while histopathology from mock-biopsy detected 55% of infected sheep. Together, these 2 tests from mock-biopsy samples detected 64% of infected sheep. This was more than twice the sensitivity of any other antemortem test at that age.

We concluded from this study that biopsy is a practical technique, about the same level of expense and difficulty as ovariohysterectomy in a large dog. It was consistently the most sensitive non-lethal technique for identification of infected sheep, although even at 36 months it detected only 2/3 of infected sheep. It may be useful as an additional tool in the management for individual valuable sheep from infected stud flocks.

Many sheep (15% in this study) recovered from early infection with *M. a. paratuberculosis*. Thus, early testing (eg at about 12 months of age), even with the most sensitive available test

(culture of biopsied tissue), may not be predictive of later infection status.

By 18 to 30 months old, positive test results for CMI, humoral immunity or faecal excretion, were often highly associated with later death from OJD, although not often with infection status at 3 years. The lack of statistical association with final infection status reflects the low sensitivity of these tests at this stage. Biopsy results at 24 months detected twice as many infected sheep as most other tests, and were very highly associated with later death from OJD.


The relatively poor performance of current diagnostic tests, and even surgical biopsy, to detect individual sheep with subclinical infection has been highlighted by this study. In 16% of the study group, subclinical infection was demonstrated only at necropsy, and all routine tests from 9 - 36 months were negative on every occasion. Even biopsy at 36 months would have detected only half of these sheep. In another 30% of sheep, mild subclinical infection was found at necropsy, but the sheep had been detected by routine antemortem testing. However, positive results in these tests were inconsistent and often only on single occasions. Another 10% of sheep were subclinically infected but had severe histological lesions. Antemortem tests performed better in these sheep. All were detected by faecal examination, mostly at the 36 month test, but most were positive in immunological tests only on single occasions. Antemortem tests performed best in the 15% of sheep that died of OJD, with all having positive IFC results prior to death.

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1. BACKGROUND AND INDUSTRY CONTEXT

Most of the tests used in the OJD program are flock tests. Individual tests are normally lethal tests such as histopathology and tissue culture, and have mostly been used as follow-up tests as part of flock evaluation. Recently, serial individual faecal cultures have been used to in an attempt to determine whether valuable individual sheep are excreting *M. a. paratuberculosis*. However, due to the insidious nature of the disease, in particular the very long incubation period and the delay in faecal shedding and seroconversion, any individual animal testing is usually considered to be impractical and unreliable.²⁶ There is a need for an accurate test or testing strategy for individual animals, particularly where it is desired to show with confidence that a given individual is not infected. The detection of OJD on studs is one example where individual animal testing is desired. Trace forward investigations are another example where it is of critical importance to establish whether imported individual sheep are infected or uninfected. It may be possible to provide, as part of a risk assessment, a classification for individual sheep from within an infected flock. This is so because it is known that OJD affects only a proportion of animals within an infected flock. Some animals, although exposed to the infection, either do not become infected, or eliminate the infection, and pose no risk to other livestock. The evidence for this comes from experiments in the UK where sheep^{2 6} or cattle^{8 21} apparently recovered from infection, and from on farm observations in Australia.²⁶

In order to exploit existing tests and develop new strategies for their use in individual animals, greater understanding of the progression of the infection in individuals was required as well as better knowledge about the behaviour of tests at different stages of the disease.

The current investigation was a longitudinal study, where a group of animals was studied in detail for several years. New tests, such as gamma interferon, and biopsy could be evaluated to great advantage. Several previous studies have shown promising results for intestinal biopsy for paratuberculosis in cattle,^{1 13} but it is not commonly used. A single study with small numbers of sheep over 25 years ago provided the first hard evidence for recovery from *M. a. paratuberculosis* infection in this species.⁶

The field evaluation of the tracer weaner concept had been undertaken on the same heavily infected property and the aims and outcomes of this work, namely to detect infection within a few months of first exposure, led logically to the current trial. The two studies were complementary, particularly as homebred sheep from the second year of the tracer studies were age cohorts and mostly grazed together with the sheep from the current study. Together, they provided a picture covering the entire disease process from infection to clinical phase. The tracer weaner trials were of short duration and involved fewer tests, but provided critical information about the first months of the infection. This trial was of longer duration, involved more detailed study of later stages of infection and allowed comparison of more tests.

The results of tests, in particular intestinal biopsies, at each time point were used to define the progression of disease in each animal and the correlation of test results with the infection status at 3 years of age. This was the first study of this kind in sheep anywhere in the world.

2. PROJECT OBJECTIVES

1. Evaluate testing strategies for individual sheep at different stages of the disease
2. Provide information about the best tests to use in different age classes of sheep
3. Evaluate tests for early diagnosis of OJD
4. Assess the predictive value of early test results for later infection status
5. Observe progression of infection and possible recovery in individual sheep using biopsy of gut tissues
6. Provide preliminary data on the value of gut biopsies for early diagnosis

3. METHODS

3.1. Animals

This trial involved 77 fine-wool Merino sheep on an OJD affected property near Goulburn, NSW. These sheep were born in September 2000, and necropsied at three years of age in September 2003. OJD vaccination of lambs on the farm had commenced in September 2000, but this mob was left unvaccinated for this research. We had been working on this farm for several years and had a good understanding of infection rates in the flock. Each year approximately 10% of unvaccinated 3-year-old sheep were positive in the agar gel immunodiffusion test (AGID) and mortalities due to OJD occurred in this age class. Early results of field evaluation of the tracer weaner concept indicated that about 20% of sheep had intestinal infection detectable by culture of intestinal tissues at 6 months of age following first exposure at weaning. This enabled manageable numbers of sheep to be used in the current trial. Trial sheep (and the remainder of the sheep from the tracer weaner study) were grazed with 150 3-year-old ewes, about 10% of which were AGID positive, thus an ongoing high level of exposure to *M. a. paratuberculosis* was likely. The sheep were run under normal farm conditions on unimproved pasture, except that ewes were not joined. They received no supplementary feeding during the trial. Each lamb was examined using a range of tests at regular intervals. This regimen is shown in Table 1. In addition, the sheep were examined closely at least weekly by the farmer, and weights and condition scores were recorded at the time of each biopsy examination.

Table 1. Testing regimen.

Test	Approximate age at sampling (months)					
	9 Apr 01	12 Sep 01	18 Mar 02	24 Sep 02	30 Mar 03	36 Sep 03
Clinical exam	+	+	+	+	+	+
Faeces culture	+	+	+	+	+	+
Faeces D-PCR	+	+	+	+	+	+
IFN-g	+	+	+	+	+	+
Skin test				+	+	+
AGID	+	+	+	+	+	+
ELISA			+	+	+	+
Biopsy samples ^a		+	+ ^b	+		+ ^c
Necropsy exam						+ ^d

^a Ileum and mesenteric lymph node for culture and histopathology

^b Sub-sample of 20 sheep, based on infection at previous sampling

^c These were mock biopsies collected at necropsy examination

^d Sheep with severe clinical disease were euthanased and necropsied earlier

3.2. Clinical examinations

The clinical signs of Johne's disease are non-specific, and are emaciation, with or without diarrhoea. In the terminal stages, sheep are weak, and may have ventral oedema. In this trial any sheep in obviously poorer condition than the rest of the flock was considered to have clinical signs consistent with OJD, and was observed closely by the farmer. Whenever it was considered that the sheep was suffering or likely to die in the next few weeks, the animal was euthanased and necropsied. The usual criteria were weakness and failure to keep up with the mob.

3.3. AGID 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 °C. Serum was removed within 48 hours for testing in an AGID test.⁴ Results were recorded as negative, inconclusive, or positive (1+, 2+ or 3+).

3.4. Elisa test

Serum was collected and stored as above, then transported at 4°C to CSL for testing in an enzyme-linked immunosorbent assay using a commercial kit (Parachek Johne's Absorbed EIA, CSL, Parkville, Victoria). Samples were recorded as positive if sample OD exceeded control negative OD by more than 0.2.

3.5. IFN- γ testing

Blood was collected from the jugular vein into lithium heparin vacutainers, and held at room temperature for less than 12 hours prior to processing using a commercial test kit (Bovigam, Bovine gamma interferon test, CSL, Parkville, Victoria). For each sample, three 1.5 mL aliquots of well mixed blood were incubated for 18 hours at 37 °C with 100 μ L of Johnin PPD (300 μ g/mL, CSL, Lot # 0404-41101), Avian PPD (300 μ g/mL, CSL, Lot # 01301) 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 transported overnight at 4 °C to CSL for the enzyme immunoassay. Results were assessed using the manufacturer's recommended criteria. Only when OD (Johnin) was > 0.05 above both OD (PBS) and OD (Avian PPD) was the result was recorded as positive.

3.6. Skin test for delayed type hypersensitivity

Sheep were injected intradermally on the wool-free inner thigh with 0.1 mL of avian tuberculin 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. An increase in skin thickness of 4mm or more was recorded as positive.¹⁵

3.7. Faecal culture

Faecal samples were held at 4°C and processed within 48 hours, or frozen at -80 °C for up to 3 months until processed routinely.²⁴ Bactec vials were incubated for 12 weeks, and the presence of *M. a. paratuberculosis* was confirmed by IS900 PCR and REA.^{5,24} Gram-stained smears were prepared from selected growth index (GI) positive Bactec vials which were also subcultured

onto modified 7H10 Middlebrook agar,²³ with and without Mycobactin J to indicate contaminating organisms.

3.8. Direct faecal PCR

This was done as previously described.⁹ Briefly, 200mg of faeces was mixed with 700 μ L PBS in an Eppendorf tube. To extract the DNA, the tube was heated at 55 °C for 30 min, then vortexed for 3 min, before boiling at 105 °C for 30 min. Tubes were then centrifuged at 12,500g for 5 min. DNA was then purified from 300 μ L of the supernatant using a silica-resin based method (Promega-Wizard™ PCR Preps DNA purification system – Cat No. A7170). 5 μ L of the extracted DNA was used in an IS900 PCR reaction, with forward primer IS900/150C¹¹ and reverse primer P91,²² followed by REA.

3.9. Biopsy of intestine and lymph node¹⁰

On each occasion the animals were yarded, transported for about 35 km and housed in an enclosed shed with access to water. They were fasted for 12 to 24 hours before surgery. Prior to anaesthetic induction each animal was weighed. Following anaesthetic induction antimicrobial therapy was administered (20 mg/kg of long acting oxytetracycline injected subcutaneously, Bivatop 200, 200 mg/mL, Boehringer Ingelheim). At the same time, 10 mg (approximately 0.35 mg/kg) of meloxicam (Metacam, 5mg/mL, Boehringer Ingelheim) was administered subcutaneously for analgesia. The antimicrobial dose was repeated 2 days later on the morning the sheep were returned to their paddock.

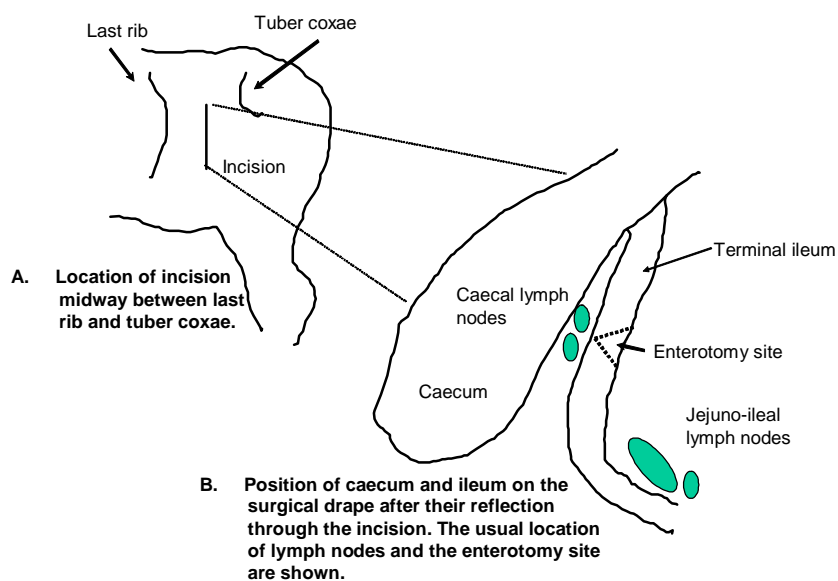
Anaesthetic induction was accomplished using an IV (jugular) injection of 5% thiopentone sodium (Thiobarb, Jurox) to effect. The dose rate was approximately 20 mg/kg and ranged from 8 mL to 25 mL per sheep (average 12.6 mL). The dose depended on the weight of the sheep and whether or not additional doses were required to maintain depth of anaesthesia during a difficult intubation. During induction two assistants held the animal in lateral recumbency on a table. Intubation with an 8.5 mm endotracheal tube was also achieved with the sheep in lateral recumbency. This size tube fit all sheep in this weight range and was placed either blindly or with the aid of a laryngoscope. Anaesthesia was maintained using gaseous halothane (2-4%) in 100% oxygen administered through a semi-closed circle machine with a Fluotec 3 vaporizer (Cyprane). A pulse oximeter and a respiratory monitor were used to observe pO₂, heart rate, and respiration during surgery. Depth of anaesthesia was monitored via palpebral reflex, response to surgical intervention, and fluctuations in the observed vital parameters.

Sheep were placed in right lateral recumbency with the head of the animal lowered over the side of the surgery table to allow saliva and ruminal reflux to drain freely into a collection bowl. Wool was clipped to skin level over the left paralumbar fossa from approximately the last rib to the tuber coxae and the lumbar vertebral transverse processes to the ventral flank. The surgical site was prepared with ether, alcohol and iodine scrubs. Surgery was performed on a small animal operating table under an operating theatre light using full sterile procedure, including a sterile pack for each animal. A left flank incision was made midway between the last rib and the tuber coxae and approx. 7.5 cm ventral to the lumbar vertebral transverse processes (Figure 1A). Initially the incision length was such that a hand could be completely inserted into the abdomen. As experience was gained with the approach the caecum could be found with 2 fingers and the incision required was much smaller (approx. 6 cm). This left flank approach had been rehearsed on freshly euthanased sheep from the tracer weaner investigations on the same farm, before being used on live animals.

The caecum was located by palpation, usually below and behind the caudal margin of the rumen, and exteriorised to expose the ileocaecal valve (ICV) and associated regional lymph

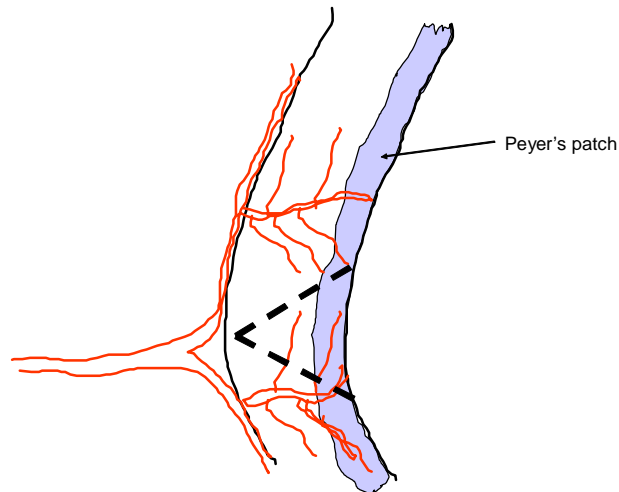
nodes (Figure 1B). It was found that regional lymph nodes were variable in position, size, and surgical availability. Lymph node tissue was always found, however, and typically only a section of the node was excised to achieve a sample approximately 1 cm³. On some occasions this necessitated removing the whole node. Often there was sufficient haemorrhage from the incised lymph node to warrant suturing the node capsule together with one or two simple interrupted or a single cruciate suture using 2/0 chromic catgut (Dynak) or 4/0 braided polyglactin 910 (Vicryl, Ethicon). Most of the lymph node sample was placed in a sterile container for culture and held at 4°C after a small (2 mm) full thickness section was cut from it and placed in 10% neutral buffered formalin for histopathology.

Figure 1. Surgical site



Following removal of as much of the intestinal content as possible by massaging it away from the desired surgical site, bowel forceps were placed in two places 5 cm apart on the ileum as close to the ileocaecal valve as was practical. This allowed a "V" shaped piece of intestine to be resected between the forceps 5-10 cm from the ileocaecal valve. The apex of the section was toward the mesenteric border but not through to the mesenteric attachment. The base of the section was 1-1.5 cm long at the anti-mesenteric margin of the ileum (Figure 2) and included a section of Peyer's patch located along the ileum. A piece of the sample (approx 2 mm) was cut from one side of the section and placed with the lymph node section in fixative for histopathology while the remaining larger portion of bowel was placed in a separate sterile container for culture and held at 4°C.

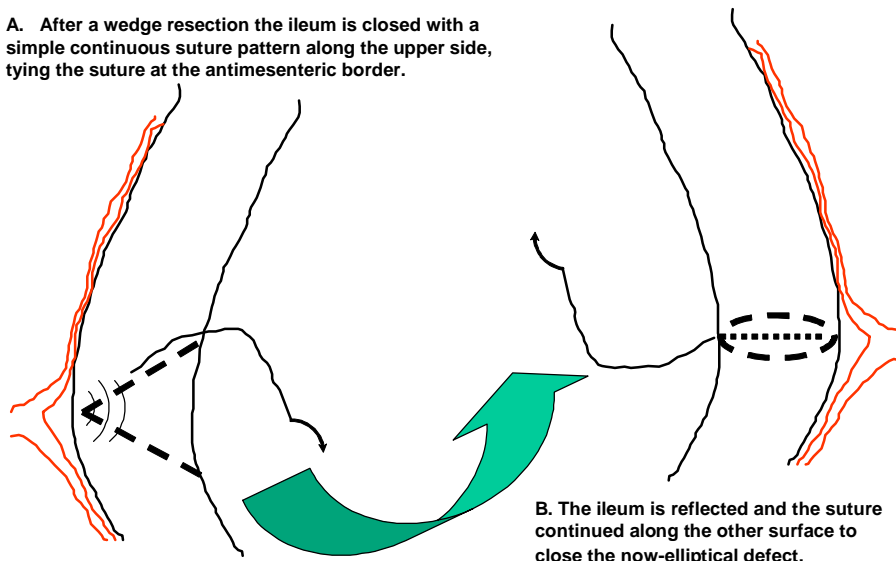
Figure 2. Location of enterotomy incisions to avoid the mesenteric border and the major vasculature.



The defect in the ileum was then closed in two layers using 4/0 Vicryl with a swaged-on tapered needle. The first layer was made in a simple continuous pattern through all layers of the bowel wall, interrupted with a tie at the anti-mesenteric border to prevent a "purse string" effect from the simple continuous pattern (Figure 3). A Cushing layer was then placed over the first layer without allowing the needle to penetrate the mucosa. The repaired defect was then checked for leaks by gently massaging ileal contents back and forth across the site. Two mL of lincomycin solution (Lincocin, 100 mg/mL, Pharmacia & Upjohn) was flushed over the ileal surgical site and into the abdomen before closure of the abdominal wall.

Figure 3. Closure of the enterotomy

A. After a wedge resection the ileum is closed with a simple continuous suture pattern along the upper side, tying the suture at the antimesenteric border.



B. The ileum is reflected and the suture continued along the other surface to close the now-elliptical defect.

The abdominal wall was closed using either 0 chromic catgut (Dytek) or 1 braided polyglycolic acid (Dexon, Davis and Geck) suture material. The peritoneum and all muscle layers were included in a simple interrupted or continuous suture pattern depending on the surgeon's preference. Occasionally the subcutaneous tissue was closed separately in a simple continuous pattern in an attempt to lessen dead space. The skin was closed with 0 polyamide suture material (Supramid, Braun), 0 nylon (Ethilon, Ethicon), or 1 polyester (Mersiline, Ethicon) in a horizontal mattress pattern.

Halothane administration was terminated approximately 5 minutes before surgery was completed, with extubation following the return of the gag reflex an average of 7 minutes after completion of surgery. During anaesthetic recovery sheep were placed in large newspaper-lined cages within the heated veterinary clinic. Their heads were initially lowered over the side of the cage to minimise fluid secretions entering the trachea until the animal was swallowing well or could sit. Soon after the sheep were able to stand and walk following surgery they were placed in an enclosed shed with a concrete floor and given access to water. They were given lucerne hay the day after surgery and remained in the shed until the following morning when the second dose of oxytetracycline was administered.

Surgical wounds and animal behaviour were monitored by the farmer for a few days after the sheep were returned to the farm. They were returned to unimproved pasture without supplementary feed. Sutures were removed from all sheep at convenience and up to 10 weeks post surgery.

Weight measurements were recorded at the time of surgeries and were used to determine live-weight gains of the sheep over the course of the trial. Weights recorded at the time of the third round of surgeries were used to compare weight changes of animals that had undergone two previous surgeries relative to those that had only one previous surgery. A T-test was used for statistical comparison of mean differences in live-weight gains between the two groups.

3.10. Necropsy examination

All surviving sheep were killed at 3 years of age using intravenous barbiturate. Throughout the trial, sheep with severe clinical signs, were similarly euthanased. Sheep were examined for gross lesions suggestive of OJD (ileal thickening, enlarged mesenteric lymph nodes (MLN), and lymphangitis). Adhesions secondary to the biopsy procedures were recorded. Terminal ileum (TI, a pool of three segments about 100, 200 and 300 mm proximal to the ICV), ICV, and MLN (a pool of segments from three nodes, including an ICV and caudal jejunal node if possible) were collected into sterile containers for culture. Adjacent tissues from the same sites were collected into 10% buffered neutral formalin for histopathology.

3.11. Culture of tissues

Tissue samples were held at 4°C and processed within 24 hours of collection, or frozen at -80°C and processed within 3 months. Tissues from biopsy were split into two aliquots and processed in parallel using a standard technique²⁴ and a modification including a centrifugation step which provides increased sensitivity.¹⁷ Tissue samples from necropsy were processed by the centrifugation method only. In addition to the separate cultures of TI, MLN and ICV, homogenate pooled from the three tissues from each sheep was cultured.¹⁸ Subsequent Bactec culture was as above for faeces.

3.12. Histopathological examination

Tissues were processed routinely for histopathology, sectioned at 5 µM, stained with

haematoxylin and eosin and a Ziehl-Neelsen technique, and examined by light microscopy. Sheep were classified as histologically positive for Johne's disease when typical focal, multifocal or diffuse lesions consistent with OJD were seen.^{3 14} The minimum criterion for a positive result was the finding of at least two clumps of macrophages with typical epithelioid morphology in a usual predilection site, with or without the presence of acid-fast bacilli (AFB). An equivocal result was recorded when no AFB were detected, and isolated clumps of cells with morphology indicative, but not typical of the macrophages usually associated with OJD, or granulomas indicative of OJD, but more consistent with another aetiology, were present. Macrophages were considered atypical if they contained refractive or pigmented material. Granulomas were considered more consistent with another aetiology if they had mineralised contents, contained material indicative of vegetable matter or the possible remnants of parasites, or if there were large numbers of eosinophils within the granuloma.

Lesions were scored using the classification system of Perez¹⁴ with an additional category (2n) for animals with lesions only mesenteric lymph nodes as follows:

- 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, architecture preserved
- 3b Diffuse lesions, multibacillary, architecture is disrupted (villous blunting, fewer crypts etc)
- 3c Diffuse lesions, paucibacillary (other inflammatory changes more prominent than macrophage infiltration)

In addition, the presence of acid-fast bacilli was scored as: 0 (none), + (occasional, usually single, and need to search many fields to detect), ++ (small to moderate numbers, often clumped and present in most fields with lesions), +++ (abundant, often diffuse and readily seen from low power examination of sections).

3.13. Classification of sheep with regard to infection status

A sheep was classified as infected at a particular sampling time if it had direct evidence of *M. a. paratuberculosis* infection, viz. a positive faecal or tissue culture, positive D-PCR or histological lesions consistent with OJD. If all these tests were negative, the sheep was classified as uninfected at that sampling. Reactions in immunological tests alone were not considered sufficient to classify an animal as infected.

A final infection and disease status was assigned to each sheep after necropsy as follows:

Uninfected sheep were further classified as

Never infected (not detected to be infected at any sampling)

Recovered (uninfected at necropsy but infected at a previous sampling).

Infected sheep were further classified as

Died of OJD (euthanased with severe clinical disease, confirmed by laboratory testing)

Subclinical OJD (euthanased at 36 months, infected, but without clinical signs).

The sensitivity, specificity and positive and negative predictive values of each test at each time, relative to the final infection status, were calculated. Test results (positive or negative) at antemortem samplings were compared with final status (infected or uninfected, and died or not died of OJD) using the chi

square test on 2x2 contingency tables. The Fisher exact test was used when an expected cell value was less than 5 and the chi square results were likely to be invalid.

3.14. Assessment of the sensitivity of biopsy at different stages of disease

Mock-biopsy tissue samples, identical to those which would have been removed at surgery, were collected at necropsy from sheep in several separate trials on the same property. The culture and histopathology results from mock-biopsy tissues (node and ileum) for four groups of sheep at different stages of the disease were compared to the results obtained from culture and histopathology from the full range of necropsy tissues using McNemar's Chi-square test for paired observations.¹²

Early infection in mixed age sheep (up to 12 months post-exposure) – 66 clinically normal sheep that had been naturally exposed to *M. a. paratuberculosis* for 5 to 12 months during the tracer weaner study.¹⁵

Early infection from birth (12 months of age) – A subgroup of 17 of the 66 sheep above that were exact age and exposure cohorts (ie exposed from birth and necropsied at 12 months of age) as the sheep in the current trial.

Clinically normal 3 year old sheep exposed since birth – 63 clinically normal sheep from the current trial that were euthanased at 3 years of age.

Severe/fatal clinical OJD - Seven sheep from the current trial that were euthanased with severe clinical OJD.

3.15. Comparison of culture and histopathology of tissues for OJD detection at necropsy

The current trial, combined with the tracer weaner studies¹⁵ and ewe-lamb investigations⁷ on the same farm, provided an opportunity to assess the relative ability of culture or histopathology to detect infection at necropsy in sheep of different ages that had been heavily exposed to *M. a. paratuberculosis* since birth. McNemar's Chi-square test for paired observations was used.¹²

4. RESULTS AND DISCUSSION

4.1. Biopsy surgery

The sheep tolerated handling and transport without obvious signs of distress. In an effort to minimize the stress from the clinical environment they were yarded outside the clinic building and only brought inside at the time of anaesthetic induction. As soon as they were capable of standing and walking following surgery they were removed from the clinic to the outside holding shed. They remained quiet and easy to manage in the clinical environment.

A total of 170 laparotomies were performed by five veterinarians. Details of the surgeries for each sheep are given in Appendix 1. The anaesthetic induction and surgical site preparation time was approximately 10 minutes per animal. Intubation difficulties were experienced in some sheep and required increasing the depth of anaesthesia by administration of additional thiopentone sodium. Surgical operating time was from 30 to 87 minutes (mean duration 46 minutes). The animals were extubated approximately 7 minutes after the surgery was completed and were standing from as little as 15 minutes up to over 3 hours following extubation.

The enterotomy itself took up to 30 minutes and was the most complicated and time-consuming part of the procedure. The distal portion of the ileum was easy to exteriorise by reflecting the caecum through the incision in the abdominal wall. The ileal biopsy site was approximately 5-10

cm from the ileocaecal valve. The biopsy was a wedge shaped piece of tissue with a 1-1.5 cm length at the antimesenteric border that narrowed to a point at the mesenteric border. Care was taken to avoid cutting through the mesenteric attachment so that the integrity of the blood supply at the anti-mesenteric border was not compromised and a reference point for anastomosis remained. Haemorrhage was unavoidable but not significant and there was no need to ligate ileal vessels. Although ileal contents were milked from the biopsy site prior to opening the bowel, minimal spillage of digesta did occasionally occur during the procedure. This viscid, deep green material was removed using gauze swabs. Although bowel that had been biopsied previously often had a slight fibrous scar or rarely minor mesenteric adhesions, there was little interference with surgical procedures. Adhesions were generally easily broken down and there was no difficulty avoiding a previous scar while taking another biopsy.

The use of continuous suture patterns in the ileal surgery, as opposed to single, crushing (Gambee) sutures, allowed a relatively fast closure of the defect left by the biopsy and has led to no apparent negative sequelae for the animals. 4/0 Vicryl with a swaged-on needle was easy to use, atraumatic, pliable, and had good knot security. The wedge shape of the biopsy allowed an adequate appositional length so as to avoid undue luminal narrowing. The external diameter of the bowel at the suture site post closure was similar to that of adjacent ileum and no leakage from the repaired defect was observed in any sheep.

The major difficulty in taking a lymph node biopsy was variability in the size and locations of nodes near the ileo-caecal junction. The larger nodes haemorrhaged more and required one or two deep sutures to appose the edges of the cortices for adequate haemostasis. In the case of small nodes removed en bloc haemorrhage was minimal.

There were few signs of pain and no sign of post-surgical ileus. All sheep took water and food in the days after surgery and their behaviour soon after returning to the farm was not distinguishable by the farmer from that of other sheep. The only post surgical complication reported from "Hillwood" involved a slight lameness in the left hind leg for up to a week post surgery in some animals. Feeding patterns and body weight were not obviously different from other sheep at the time of initial suture removal or at shearing, which was 6 months after the initial surgery.

Weight measurements taken at the time of the third surgery demonstrated that those sheep that had undergone surgery twice had gained an average of 2.4 kg between the 12 and 24 month examinations, whereas sheep that had only one surgery had gained an average of 4.7 kg ($p < 0.01$). This difference is more likely to be an effect of subclinical OJD, than an effect of additional surgery, since sheep were selected for biopsy at 18 months based on having been identified as infected at previous sampling. When returned to "Hillwood", the sheep subjected to the surgeries were managed as a mixed flock with other sheep of the same age. The farm manager felt that overall the sheep subjected to the surgeries appeared to perform and compete at least as well as other sheep on the property, although the latter group was not weighed for comparison.

The samples collected at biopsy were adequate for culture and histological assessment of paratuberculosis.

4.2. Clinical signs

Over the course of the trial, 11 sheep were euthanased with severe OJD. All except one had deteriorated rapidly, and were euthanased within a month of first showing clinical signs. Sheep 54 had shown clinical signs for 6 months, before it developed significant weakness and had to be euthanased. No other sheep displayed clinical signs. The condition of the animals varied with the pasture conditions. During summer 2002–2003, feed was not abundant and the

animals were in light condition (average condition score of only 1.9). Five animals were scored as CS 1, but were otherwise very robust, and, considering the average condition of the flock, were not considered clinical cases. Over the course of the trial, three sheep died in the paddock and were not necropsied. Two of the deaths were associated with a sudden change of weather off-shears. Although two of them had confirmed OJD infection at their last sample, one with severe lesions and one with a positive faecal culture, they could not be assigned a final OJD status, and were excluded from statistical analysis. The remaining 63 sheep, all clinically normal at the time, were finally necropsied at 36 months, and a total of 74 sheep were available for detailed analysis of test performance.

4.3. Overview of the final infection status of each sheep

The numbers of individual sheep classified by final OJD status are given in Table 2. The detailed results for each of the tests applied for each sheep at each sampling time are given in Appendix 2.

Table 2. Classification of 74 sheep by final OJD status

Final OJD status	Number of sheep	%	Comment
Uninfected			
<i>Never infected</i>	16	22	5 with immune reactions ^a
<i>Recovered</i>	11	15	7 with immune reactions
Total uninfected	27	36	
Infected			
<i>Subclinical OJD</i>	36	49	6 severe ^b
<i>Died OJD</i>	11	15	
Total infected	47	64	

^a a positive result on at least one occasion in the Elisa, AGID, IFN-g or skin test

^b severe diffuse lesions on histopathology

Sheep that were never infected – The majority of these sheep (11/16) never reacted in any test. Note, however, that these were as heavily exposed to *M. a. paratuberculosis* as the rest of the trial sheep, and may well be among the most genetically resistant sheep. (Samples from this trial have been stored for possible future genetic analysis.) Presumably, infection was never established at a level which could be detected with the means available in this trial, and infection was controlled or eliminated without the production of detectable adaptive immune responses.

Five sheep reacted in an immunological test but never had direct evidence of infection (Table 3). All were positive only to tests for CMI, being consistently negative in all tests for humoral immunity. All but one of the sheep had a positive result on only one occasion. Three (12, 69 and 90) had a single positive result in the IFN- γ test at 30 mths, when 48% of sheep tested were positive, and Sheep 81 had a positive skin test only at 24 months, when 32% of sheep were positive. Only Sheep 32 was positive on multiple occasions in both the IFN- γ and skin tests from 24 months. As above, it is likely that these sheep were exposed to *M. a. paratuberculosis*, and infection was controlled or eliminated, but in these sheep there were detectable adaptive cell-mediated immune responses, albeit on few occasions in most.

Table 3. Uninfected sheep with immunological reactions

Sheep ID	Age when positive test result (months)			AGID
	IFN-G	DTH	ELISA	
12	30	-	-	-
32	30, 36	24,30	-	-
69	30	-	-	-
81	-	24	-	-
90	30	-	-	-

Recovered sheep – Four of the eleven recovered sheep were consistently negative in immunological testing (Table 4). Presumably, infection, although established at a detectable level, was controlled or eliminated without the production of detectable adaptive immune responses, and as above, these may be genetically very resistant sheep. An alternative explanation is possible for three of these sheep (4, 40 and 73), in which infection was diagnosed by a single faecal result (culture or D-PCR) at 9 or 12 months of age. These positive results could be passive excretion of ingested *M. a. paratuberculosis* organisms. Passive excretion has previously been demonstrated within a week after experimental oral infection of lambs with a single dose of 10^7 viable organisms.¹⁹ Such a dose would be ingested by a sheep ingesting a fraction of a gram of fresh faeces from a multibacillary infected sheep.²⁵ Trial sheep were grazing with heavily infected adults, and pasture on the farm in winter/spring 2002 was very short. This may have facilitated the ingestion of faecal material. Also, as discussed later, D-PCR may detect ingested non-viable organisms from pasture or soil. The fourth sheep (51) was IFC positive at 9 months and histologically positive at 18 months.

Table 4. Recovered sheep with no immunological reactions

Sheep ID	Age when positive test result (months)			
	IFC	D-PCR	Biopsy culture	Biopsy histo
4	-	9	-	-
40	-	12	-	-
51	9	-	-	18
73	9	-	-	-

Seven of the eleven recovered sheep also had positive results in some immunological tests (Table 5). All were positive at least once in tests for CMI, and three also had detectable humoral immune reactivity.

Table 5. Recovered sheep with immunological reactions

Sheep ID	Age when positive test result (months)							
	IFC	D-PCR	Biopsy result		IFN-G	DTH	ELISA	AGID
			Culture	Histo				
1	-	-	12	-	24,30	24	24	-
7	9	-	12	24	-	24,30	-	-
30	12	-	12,18	12,18	18,30,36	24	18,24	18,30
52	-	-	-	24	30,36	-	-	-
57	-	-	-	24	-	24	-	-
64	-	-	24	-	30	-	-	-
72	9	9	12	-	9,30	24	24	-

Sheep with subclinical OJD at 36 months – More than half (36) of the surviving sheep were in this category.

Ten of these sheep (all classified as having mild OJD) were detected only by examination of samples collected at necropsy at 36 months of age. All other tests, both direct and

immunological, were negative at all time points (Table 6). All but one sheep (positive only in the histology on the mock-biopsy sample) were detected by culture of necropsy tissues. Four sheep had positive results from the mock-biopsy sample – ie. they would have been detected in a biopsy examination at this age. Five sheep would have remained undetected using the whole of the antemortem sampling regimen of this trial (repeated tests for infection and immune response, including biopsies, from 9 to 36 months of age). This represents 5/63 or 8% of surviving sheep in the flock. If biopsy is not included, the figure for undetected infection after repeated testing with all routine antemortem tests rises to 9/63 or 14%.

Table 6. Infected sheep, detected only at necropsy. All other tests negative

Sheep ID	Full necropsy ^a		Mock biopsy ^b		status
	Culture	Histo ^c	Culture	Histo ^c	
6	+	-	-	-	mild OJD
14	+	+(1)	+	+(1)	mild OJD
16	+	+(1)	+	+(1)	mild OJD
26	+	+(2)	+	+(2)	mild OJD
43	+	+(2n)	-	-	mild OJD
47	-	-	-	+(1)	mild OJD
62	+	-	-	-	mild OJD
66	+	-	-	-	mild OJD
67	+	-	-	-	mild OJD
79	+	-	-	-	mild OJD

^aSamples (each ~2.0g) from 3 sites in terminal ileum, 3 mesenteric lymph nodes and ileocaecal valve

^bSamples (each ~0.5g) from single site in terminal ileum and single mesenteric lymph node

^cLesion classification shown in parentheses

A further 20 sheep had mild OJD detected by necropsy examination at 36 months, but had also been positive in antemortem testing on at least one occasion in at least one test (Table 7). Only six of these sheep had direct evidence of infection from faecal examination (IFC or D-PCR), and only on one or two of the six sampling times. Repeated biopsy would have detected 15, 13 of these at the 36 month mock-biopsy. 19 of the 20 sheep had been positive in at least one immunological test, but 11 only in one test on a single occasion. Only 3 sheep were repeatedly positive in immunological tests – sheep 27 in the IFN- γ test, and sheep 63 and 65 in the ELISA.

Table 7. Sheep with mild OJD at necropsy, positive results in antemortem tests

Sheep ID	Age when positive test result (months)								Necropsy results	
	IFC	D-PCR	Biopsy results		IFN-G	DTH	ELISA	AGID	Culture	Histo ^a
2	-	12	-	24,36	-	24	-	-	-	+(1)
5	36	36	36	36	-	-	30	-	+	+(2)
19	-	-	-	36	-	30	-	-	+	+(1)
23	30,36	30,36	12,36	36	30,36	30	-	-	+	+(3a)
27	36	36	12,18,24,36	18,24,36	9,18,24,30,36	24,30	36	-	+	+(2)
31	-	-	36	36	30,36	24	-	-	+	+(1)
35	-	-	12	24	30	24	-	-	-	+(1)
36	-	-	-	-	30	-	-	-	+	+(2n)
37	-	-	36	36	30	-	-	-	+	+(2n)
39	-	-	36	36	30	-	-	-	+	-
41	-	-	-	-	30	-	-	-	+	-
42	-	-	36	-	30	24	-	-	+	-
44	-	-	-	-	30	-	-	-	+	-
56	-	-	-	-	30	-	-	-	+	+(2n)
61	-	-	-	36	24,30	-	-	-	+	+(1)
63	-	-	12,36	12	9,30	24	18,24,30,36	-	+	-
65	-	-	12,18	-	-	24,30	18,24,30	-	+	-
70	-	-	12,36	-	36	-	-	-	+	-
77	9	-	-	-	-	-	-	-	+	-
80	-	12	36	36	30	-	-	-	+	+(2)

^aLesion classification shown in parentheses

Six subclinically affected sheep were considered to have severe OJD infection, based on having severe, diffuse histological lesions at necropsy (Table 8). Three sheep had severe diffuse paucibacillary lesions, and three had mixed lesions. All were detected by faecal examination, mostly at the 36 month test, but one only at the 9 month test. All would have been detected by biopsy at 36 months, and half were detected in an earlier biopsy. All were positive in the IFN- γ test, but four only on one occasion at 30 months. Four were positive in skin testing, but three of these only on a single occasion. ELISA and AGID tests were positive for only three sheep each, and all on only a single occasion.

Table 8. Sheep with subclinical OJD, but with severe diffuse lesions, at necropsy

Sheep ID	Age when positive test result (months)								Necropsy results	
	IFC	D-PCR	Biopsy results		IFN-G	DTH	ELISA	AGID	Culture	Histo ^a
15	30,36	24,36	24,36	24,36	30	24	-	-	+	+(3c)
17	36	36	36	36	30	36	36	-	+	+(3c)
49	36	36	36	36	30,36	24	36	36	+	+(3b/3c)
50	9	-	18,36	18,24,36	9,18,24,30,36	24,30,36	24	30	+	+(3c)
60	30,36	36	24,36	36	30	-	-	36	+	+(3b/3c)
71	36	36	36	36	30	-	-	-	+	+(3b/3c)

^aLesion classification shown in parentheses

Sheep which were euthanased with severe clinical OJD – There were 11 of these sheep (Table 9). The majority (73%) of these sheep had severe diffuse multibacillary pathology. All had positive IFC results prior to death, most on more than one occasion. Ten of these sheep had a positive IFN- γ result prior to death, but five of these only on a single occasion. Six sheep had positive DTH reactions, but five only on a single occasion. Most had positive tests for humoral antibody at the time of euthanasia, but only 5 were positive in an earlier test. Mock-biopsies at necropsy were done on only 7 of these sheep (Appendices 3c, 3d) and the culture and histopathology findings were identical to the full necropsy findings. All but one sheep was confirmed to be infected at an earlier biopsy examination, and three sheep demonstrated progression of lesions from multifocal or paucibacillary at the last biopsy to multibacillary at death.

Table 9. Sheep euthanased with severe clinical OJD

Sheep ID	Age when positive test result (months)								Necropsy results		Age at death
	IFC	D-PCR	Biopsy results		IFN-G	DTH	ELISA	AGID	Culture	Histo ^a	
8	18,24	18,24	12,18	18(3b)	18	-	18	24	+	+(3b)	24
10	30	30	24	24(3c)	18,30,35	24	18,24,30,35	24,30,35	+	+(3c)	35
13	18,24	18,24	24	24(3b)	24	-	29	29	+	+(3b)	29
29	18,24	18,24	24	24(3b)	9,18	24	24,29	29	+	+(3b)	29
46	24,30	30	-	24(3a)	30	24,30	30,33	-	+	+(3b)	33
54	9,24	-	12,18,24	12,18,24(3c)	18	-	18,24	18,24,28	+	+(3a/3c)	28
55	18,24	24	-	-	-	-	24	24	+	+(3b)	24
58	24,30	24	12,24	24(3c)	24,30,34	24	34	30,34	+	+(3b)	34
74	24	24	24	24(3b)	24	24	-	28	+	+(3b)	28
76	9,18	9	12,18	12,18(3c)	9,12,18,24	-	24	-	+	+(3c)	24
78	18,24	24	12,18,24	18,24(3b/3c)	18,24	24	-	29	+	+(3b)	29

^aLesion classification shown in parentheses

4.4. Overview of the performance of each test for OJD

Table 10 shows the percentage of surviving sheep at each sampling time which were positive in each of the tests applied. Summary test results for each sheep are given in Appendix 3, and statistical analyses of the predictive values of each of the tests for final infection status, are given in Appendix 4, along with sensitivity and specificity relative to final infection status.

Table 10. Test results at each sampling time.

(Percentage of surviving sheep positive for each test)

	Age at sampling (months)					
	9	12	18	24	30	36
Clinical samples						
Faeces culture	10	1	9	12	9	14
Faeces D-pcr	4	4	4	11	5	14
IFN-g	8	1	13	11	48	14
Skin test	ns	ns	ns	32	12	3
AGID	0	0	3	3	6	3
ELISA	ns	ns	8	12	8	6
Biopsy samples						
Tissue culture	ns	19	40 ^a	20 ^b	ns	30 ^c
Histopathology	ns	5	40 ^a	23	ns	32 ^c
Combined	ns	19	45 ^a	26	ns	37 ^c
Necropsy exam						
Combined						57
Total sheep	77	77	77	74	66	63

^a Result from only 20 sheep, based on infection at previous samplings

^b Result from only 60 sheep, contamination of batch of Bactecs

^c These are results for mock biopsies at necropsy examination

ns Not sampled

Faecal culture - At 9 months, eight (10%) sheep were positive, an unexpectedly high result, given that only a single animal was positive by IFC at 12 months. Passive excretion (associated with short winter pasture??) may partially explain these results. There was no association with

final OJD status. Four of these sheep had no evidence of infection at necropsy, although 3 of the 4 later had infection in tissue detected by later biopsy. Faecal culture status at 18, 24 or 30 months, however, was highly predictive of a fatal outcome, and specificity for final infection status was 100%. At 18 months, 7 sheep were IFC positive. One later died in paddock and was not included in analysis, and the remaining 6 all died of OJD. At 24 months, 9 were IFC positive, 2 later died in paddock and the other 7 all died of OJD. At 30 months, 6 were IFC positive; 3 died of OJD, and the other 3 were still infected at necropsy. However, culture on a single occasion was statistically associated with final infection status only at 24 months ($P < 0.05$), and greatest sensitivity was only 16% at 24 months. When serial faecal results were considered, there was significant association with final infection status, and increased sensitivity (21% for 18 and 24 month results, 25% for 24 and 30 months, and 39% for 18, 24 and 30 months). At the 36 month sampling, 9 of the surviving sheep were IFC positive (all had OJD lesions at necropsy), and 5 of these had severe diffuse OJD lesions, a highly significant association.

D-PCR – These results followed a very similar pattern to the IFC results. At most time points, however, D-PCR detected fewer infected sheep than IFC. The days for cumulative growth index in Bactec culture to reach 1000 (dcgi1000), which varies directly and inversely with log numbers of viable organisms,¹⁶ were compared to D-PCR results using student's T test. D-PCR-positive samples contained larger numbers of organisms ($P < 0.00001$), consistent with previous findings.⁹ Mean dcgi1000 of D-PCR positive faeces was 29.7, compared to 41.6 for D-PCR negative samples. This translates to a difference of about 2 logs in numbers of viable organisms.

Considering the 9 and 12 month samplings together, there were four IFC-negative samples which were D-PCR positive. In theory, D-PCR can detect *M. a. paratuberculosis* organisms which are non-viable, and so will not grow in culture. Non-viable organisms on pasture or in soil may sometimes be ingested in large amounts, and the passive excretion of these organisms in faeces may be detected by D-PCR. Two of the four sheep with D-PCR-positive/IFC-negative samples (no. 4, 40) were classified as *recovered*, rather than *never infected*, on the basis of this single positive D-PCR result.

Tests for humoral immunity - Early results in these tests were not helpful, with all sheep negative at 9 and 12 months in the AGID. Of 2 sheep that were AGID positive at 18 months, one later died of OJD and the other recovered. Positive AGID tests at 24 or 30 months were significantly associated with later death from OJD. The 2 sheep that were AGID positive at 24 months both later died of OJD, as did 2 of 4 positive at 30 months. 9 of the 11 sheep that died of OJD had a positive AGID test at necropsy, and in 6 of these this was the only positive AGID result. Positive ELISA results at 18, 24 and 30 months were associated with death from OJD, but not significantly associated with final infection status (3 of 6 positives at 18 months later died of OJD, as did 3 of 9 at 24 months, and 2 of 5 at 30 months). Of 8 sheep which were tested in the ELISA at necropsy, 7 were positive, and in 4 of those, this was the only positive ELISA result.

Tests for cell mediated immunity - Six sheep were IFN- γ positive at 9 months, but there were no significant associations of these results with final OJD status. Three of these sheep were also positive at this time by faecal culture, and all six IFN- γ positive sheep were confirmed to be infected on at least one occasion. One sheep was positive at 12 months, and it later died of OJD. Positive IFN- γ results at 18 or 24 months were highly associated with later death from OJD, but not significantly associated with final infection status (6 of 9 positives at 18mths later died of OJD, as did 4 of 8 at 24 months). Many sheep (48%) were IFN- γ positive at 30 months, and at this time there was a significant association with final infection status. At 36 months IFN- γ was not helpful to identify either infected sheep or those with severe lesions. Many sheep (32%) had positive skin tests at 24 months, and at this time, positive results were significantly

associated with later death from OJD. Few sheep reacted in the later samplings, and there were no significant associations with final status.

Results from biopsy examinations - Culture of tissues obtained by biopsy at 12 months detected many more infected sheep than any other antemortem test. There was no significant association between these results and final infection status at 3 years of age, but positive biopsy results at this time were significantly associated with later death from OJD. Histopathology at this age was not sensitive, but of the 4 histologically positive sheep, 2 later died of OJD. One was mildly affected and one had recovered at 36 months. At 18 months of age, histopathology and culture from biopsies detected the same numbers of infected sheep (and mostly the same sheep), but because only a biased sub-sample of the sheep was tested, analysis of predictive value of biopsy at this age was impossible. At 24 months, biopsy detected about twice as many infected sheep as most other tests, and biopsy results were highly predictive of fatal outcome. Unfortunately, there was contamination of a batch of the Bactec cultures at this time, which was undetected by our usual quality control procedures. Thus, meaningful culture results from biopsy were unavailable for 13 of the sheep, so the percentage of sheep positive by biopsy (based on the all surviving sheep) may be an underestimate. When only the 60 sheep with valid culture results were considered, 25% of sheep were positive by biopsy, and there was significant association with final infection status ($P < 0.01$) and with later death from OJD ($P < 0.0001$). Mock-biopsy at 36 months detected 64% of infected sheep, again more than twice the number of any other test.

4.5. Discussion of test performance, including tests in combination, at each sampling time

Less than 12 months of age – At 9 months of age, 8 sheep were faecal culture (IFC) positive, 3 were D-PCR positive (including 2 IFC positive) and 6 were IFN- γ positive (including 2 IFC and D-PCR positive and one IFC positive). Taken together, 12/77 (16%) sheep had positive results in at least one of these three tests, yielding sensitivity and specificity relative to final infection status of 0.15 and 0.81 respectively. AGID results were negative in all sheep, and ELISA and skin-testing were not performed. At this age, active excretion of *M. a. paratuberculosis* is usually considered to be unlikely. However, the finding that 3 of the 8 IFC positive sheep also had IFN- γ reactions ($\chi^2 = 10.97$, $P < 0.01$) is highly suggestive of active infection in these sheep at this time. Also, it takes some months after experimental infection for IFN- γ to be detected.²⁰ The fate of these three sheep varied – one died of OJD, one had severe lesions at necropsy at 3 years and the third sheep recovered. Two sheep had single positive results in the D-PCR or IFC only, and all subsequent test results were negative. Thus, they were classified as recovered sheep, but passive excretion of ingested organisms at this 9 month sampling was certainly a possibility.

12 months of age – At this stage, only 5 sheep (6%) had positive results in IFC (one sheep), D-PCR (three sheep), or IFN- γ (one sheep) tests, yielding sensitivity and specificity relative to final infection status of 0.06 and 0.93 respectively. AGID results were negative in all sheep, and ELISA and skin-testing were not performed. The very low sensitivity of routine tests, even in combination, at this stage was expected. Biopsy, however, was positive in 15 sheep (19%), with sensitivity and specificity of 0.23 and 0.85 respectively. It was by far the most sensitive antemortem test at this time. The relatively low specificity for biopsy at this stage is a reflection of the number of sheep which later recovered from detectable infection.

18 months of age – 13 sheep (17%) were positive when the results of all routine antemortem tests applied at this time (IFC, D-PCR, AGID, ELISA, IFN- γ) were considered together (sensitivity of 0.26, specificity of 0.96). Sensitivities of the individual tests were 0.13 (IFC), 0.04 (D-PCR), 0.02 (AGID), 0.11 (ELISA) and 0.17 (IFN- γ). Specificity of all tests were above 0.95. The combination of tests detected many more infected sheep than any single test. At this stage

D-PCR and AGID detected few sheep, and the combination of IFC, ELISA and IFN- γ performed exactly as the combination of all tests. This was a much improved performance over that at 12 months, and the combined results were statistically associated with both final infection status ($P < 0.05$) and later OJD mortality ($P < 0.001$). However, with a sensitivity of 0.26, three out of four sheep later shown to be infected remained undetected.

24 months of age – 26 sheep (37%) were positive when the results the routine antemortem tests (IFC, D-PCR, AGID, ELISA, IFN- γ , DTH) were considered together. This gave a sensitivity of 0.43, but specificity was greatly reduced to just 0.74. Sensitivities of the individual tests were 0.16 (IFC), 0.14 (D-PCR), 0.05 (AGID), 0.14 (ELISA), 0.16 (IFN- γ) and 0.36 (DTH). There was no statistical association of these combined results with final infection status (a reflection of the poor specificity at this time, which was mainly due to the DTH results). The combination of all tests except DTH had a sensitivity of 0.32 and specificity of 0.89, and positive results in this combination were associated with final infection status ($P < 0.05$). Biopsy had lower sensitivity (0.32) than the combined routine tests, but a higher specificity (0.85), and biopsy results were associated with final infection status.

30 months of age – 36 sheep (55%) were positive when the results of the routine antemortem tests (IFC, D-PCR, AGID, ELISA, IFN- γ , DTH) were considered together. This gave a sensitivity of 0.67, but specificity was even lower at 0.63. Sensitivities of the individual tests were 0.15 (IFC), 0.08 (D-PCR), 0.08 (AGID), 0.13 (ELISA), 0.59 (IFN- γ) and 0.15 (DTH). The large number of sheep which were IFN- γ positive at this time heavily influenced these results. The combined results were statistically associated with final infection status ($P < 0.05$). The sensitivity at this time was much improved over that at 18 months, but, still, one third of sheep later shown to be infected were not detected, and there were very large numbers of false positive results.

36 months of age – Only 16 sheep (25%) were positive when the results of the routine antemortem tests (IFC, D-PCR, AGID, ELISA, IFN- γ , DTH) were considered together. Sensitivity and specificity were 0.36 and 0.89 respectively. Sensitivities of the individual tests were 0.25 (IFC), 0.25 (D-PCR), 0.06 (AGID), 0.11 (ELISA), 0.17 (IFN- γ) and 0.06 (DTH). The reduction from the numbers positive at 24 or 30 months was due to reduced numbers of sheep reacting in tests for CMI, and the exclusion of eight sheep which had died of OJD. At this stage of the disease, faecal examination (IFC and D-PCR had identical results) was clearly superior to any immunological testing. Mock-biopsy at this time was almost twice as sensitive (0.64) as all other antemortem tests combined.

Overview – At no sampling time was there any test or combination of tests which was both sensitive and specific for later infection status in individual sheep. Sensitivity tended to increase with age (and later stage of disease) in all tests, but there were large variations at some sampling times, particularly in the tests for CMI. The apparently better performance of IFC and IFN- γ at 9 months compared to 12 months is interesting, but cannot yet be explained. At 12 months, biopsy was many times more sensitive than all other tests, but some biopsy-positive sheep later recovered. At 18, 24 and 30 months, IFC, ELISA and IFN- γ usually had similar performance, and a combination of these tests had similar sensitivity and specificity as biopsy at 24 months. At 36 months IFC was superior to the immunological tests, and mock-biopsy at this time was twice as sensitive as the combination of all other antemortem tests.

4.6. Sensitivity of biopsy relative to necropsy

Early infection in mixed age sheep (up to 12 months post-exposure) – Culture from biopsy samples was significantly ($P < 0.01$) less sensitive than culture of necropsy samples. 23 of 66 sheep were confirmed to be infected; 10 of these were detected by culture of mock-biopsy specimens, compared to 22 by culture in the full necropsy protocol. Histopathology on mock-

biopsy specimens was also significantly ($P < 0.05$) less sensitive than histopathology on full necropsy samples. Only 6 sheep had histological lesions and all were culture-positive; all 6 were detected by histopathology on full necropsy samples, but only one from mock-biopsy. Overall, at this early stage of infection, biopsy detected 45% of infected sheep, and histopathology detected no additional infected sheep.

Early infection from birth (12 months of age) – When the 17 sheep that were exact age and exposure cohorts as the biopsy trial sheep were considered alone, results were similar. Mock-biopsy detected 33% of the sheep shown to be infected by necropsy at 12 months of age. 9 were confirmed infected by necropsy (all 9 were culture positive, and 5 were histologically positive). Culture from mock-biopsy detected only 3 sheep ($P < 0.05$), and histopathological examination detected only one ($P < 0.05$).

Clinically normal 3 year-old sheep exposed since birth - In these sheep, culture of mock-biopsy samples was again significantly ($P < 0.001$) less sensitive than culture of samples from necropsy. 33 of the 63 sheep were culture-positive; all were detected by culture of full necropsy samples, compared to 19 by culture of mock-biopsy samples. There was no significant difference between biopsy and necropsy when histopathology was used alone. 23 sheep had histological lesions of OJD; 22 were detected by the full necropsy samples, and 20 were detected by mock-biopsy. When histopathology and culture were considered together, a total of 36 sheep were confirmed to be infected; 35 of these were detected by necropsy and 23 by mock-biopsy ($P < 0.01$). At this age, examination of biopsy samples detected 64% of infected sheep, and histopathology detected additional infected sheep.

Severe/fatal clinical OJD - Mock-biopsy and necropsy yielded identical results (all positive) for all 7 sheep that were examined (Appendices 3c and 3d), reflecting the severe diffuse nature of the lesions in the terminal stages of the disease.

Samples collected from biopsy consisted of a single piece of tissue from the terminal ileum and another from a single mesenteric lymph node. In a full necropsy examination, samples are taken from at least three sites from the terminal ileum, from three mesenteric lymph nodes, and from the ileocaecal valve, while additional sites with suggestive gross lesions may also be sampled. Thus for histopathology, at least 7 sections are examined from a necropsy examination, compared to just two from biopsy. In addition, the size of each sample collected and processed for culture from biopsy is smaller than the necropsy samples (about 0.5 -1 g from biopsy compared to about 2.0 g from necropsy). Thus it was not surprising that biopsy, and in particular culture from biopsy, was less sensitive than necropsy in detecting current infection, unless the lesions were severe and diffuse.

4.7. Comparison of culture and histopathology of tissues for detection of subclinical OJD at necropsy

The current trial, combined with the tracer weaner studies¹⁵ and ewe-lamb investigations⁷ on the same farm, provided an opportunity to assess the relative ability of culture or histopathology to detect infection at necropsy in sheep of different ages that had been heavily exposed to *M. a. paratuberculosis* since birth. Results are shown in Table 11.

Table 11. Comparison of culture and histopathology of tissues for OJD detection at necropsy

% of infected sheep detected by	Age at necropsy (months)			
	3-9 ^a	12 ^a	36 ^b	48 ^c
Histopathology	0	56	64	69
Culture	100 ^{**}	100 [*]	92 [*]	85
No. of sheep infected	10	9	36	52
(%)	(17)	(53)	(57)	(36)
Number examined	60	17	63	145

^a Tracer weaner studies^b Current trial^c Ewe-lamb studies^{**} P<0.01; ^{*} P<0.05, McNemar's chi square test for paired observations.

At <12 months of age, infected sheep were detected by culture only, and no histological lesions were present. At 12 and 36 months, culture detected significantly more infected sheep than histopathology. At 48 months there was no significant difference between the numbers detected by either test, but the combination of histopathology and culture detected significantly more infected sheep than either test used alone. Note that for the 11 sheep euthanased with severe clinical OJD, either culture or histopathology detected all sheep, reflecting the severe diffuse nature of the lesions at this stage of the disease.

Histopathology alone has traditionally been used as the “gold standard” to determine *M. a. paratuberculosis* infection status. However, the results of this study indicate that histopathology alone is inferior to culture alone if applied to animals up to 36 months of age, while at four years the combination of both tests was superior to either used alone.

5. CONCLUSIONS

Biopsy is a practical technique, about the same level of expense and difficulty as ovariohysterectomy in a large dog. It was consistently the most sensitive non-lethal technique for identification of infected sheep, although even at 36 months it detected only 2/3 of infected sheep. It may be useful as an additional tool in the management for individual valuable sheep from infected stud flocks.

Many sheep (15% in this study) recover from early infection with *M. a. paratuberculosis*. Thus, any early testing (eg at about 12 months of age), even with the most sensitive available test (culture of biopsied tissue), may not be predictive of later disease status.

By 18 to 30 months old, positive test results for CMI, humoral immunity or faecal excretion, were often highly associated with later death from OJD, although not often with infection status at 3 years. (The lack of statistical association with final infection status reflects the low sensitivity of these tests at this stage.) Biopsy results at 24 months detected twice as many infected sheep as most other tests, and were very highly associated with later death from OJD. Large numbers of sheep were DTH positive at 24 months of age and IFN- γ positive at 30 months. Poor specificity in these tests at these times would limit their use to detect infected animals, as would the lack of consistency of results from one sampling time to the next in most sheep.

The relatively poor performance of current diagnostic tests, and even surgical biopsy, to detect individual sheep with subclinical infection has been highlighted by this study. In 16% of the study group, subclinical infection was demonstrated only at necropsy, and all routine tests from

9 - 36 months were negative on every occasion. Even biopsy at 36 months would have detected only half of these sheep. In another 30% of sheep, mild subclinical infection was found at necropsy, but the sheep had been detected by routine antemortem testing. However, positive results in these tests were inconsistent and often only on single occasions. Another 10% of sheep were subclinically infected but had severe histological lesions. Antemortem tests performed better in these sheep. All were detected by faecal examination, mostly at the 36 month test, but most were positive in immunological tests only on single occasions. Antemortem tests performed best in the 15% of sheep that died of OJD, with all having positive IFC results prior to death.

The results in this trial were necessarily generated from a flock with a high prevalence of infection. As high prevalence infection is controlled, there will be more need to apply tests in flocks with lower prevalence. It is likely that the age-threshold of opportunity for use of each test will be greater in lower prevalence flocks. The stage of the disease, rather than the age of the sheep, is likely to be important. Thus in lower prevalence flocks, findings from this study in 12 month old sheep for example, may be applicable to 2-3 year old sheep.

Finally we emphasize that this project has dealt with tests for individual animals, and has underscored the difficulty of diagnosis of OJD at any stage except for severe clinical disease. At this time, there is no antemortem test or combination of tests which can provide reasonable assurance that an individual animal is not infected with *M. a. paratuberculosis*. Fortunately, efficient flock tests exist which can examine many sheep at once – pooled faecal culture and abattoir surveillance.

The authors expect that further analysis of the results from this study will be undertaken as material from this and other related investigations is prepared for scientific publication.

6. SUCCESS IN ACHIEVING OBJECTIVES

The project has successfully met its objectives, with the caveat that the findings are directly applicable only to flocks with a high prevalence of infection. When applying these findings to low prevalence flocks some assumptions are necessary - fewer sheep will reach advanced stages of disease and sheep that do progress may be older.

Objectives 1 - 4 will be considered together:

1. **Evaluate testing strategies for individual sheep at different stages of the disease**
2. **Provide information about the best tests to use in different age classes of sheep**
3. **Evaluate tests for early diagnosis of OJD**
4. **Assess the predictive value of early test results for later infection status**

All refer to the evaluation of existing antemortem tests or testing strategies in individual animals at different stages of the disease. The results from this project underscore the difficulties in early diagnosis of OJD. At no sampling time was there any test or combination of tests which was both sensitive and specific for later infection status in individual sheep. The poor performance all of the routine antemortem tests in the early stages of disease was clear. Even in combination a sensitivity of only 0.06 was achieved at 12 months of age. At this time, biopsy was the only test with any degree of sensitivity (0.23). At 18, 24 and 30 months, IFC, ELISA and IFN- γ usually had similar performance, with relatively low sensitivities, but moderate to high specificity. In combination, they performed similar to biopsy at 24 months. By 36 months IFC was the best choice among the routine tests, but mock-biopsy at this time was twice as sensitive as all routine antemortem tests in combination.

5. Observe progression of infection and possible recovery in individual sheep using biopsy of gut tissues

15% of the sheep showed evidence of recovery, and progression of lesion severity was

observed over time in several sheep.

6. Provide preliminary data on the value of gut biopsies for early diagnosis

Biopsy far outperformed all other tests, even in combination, for early diagnosis at 12 months of age. However, sensitivity was still only 0.23, and a number of biopsy positive sheep later recovered from detectable infection.

7. IMPACT ON MEAT AND LIVESTOCK INDUSTRY

This detailed prospective examination of a heavily infected flock out to 3 years of age has provided a truly unique perspective on the disease. Insights into the progression of infection in individual animals, and the ability (or inability) of our current tests to detect this, will be helpful in the interpretation of results from other research trials and in field investigations.

Examples of particular significance include the following:

A proportion of infected sheep recover from detectable infection. Thus any early test and cull program will remove some possibly genetically resistant sheep.

Even using every available antemortem test (including biopsy) on repeated occasions, many infected sheep remain undetected. There can be no guarantees that a particular sheep is clean.

Results for most tests (in particular those for CMI) are not consistent over time.

Culture from tissues is superior to histopathology in the detection of *M. a. paratuberculosis* infection at necropsy in the early stages of the disease.

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9. APPENDICES

Appendix 1. Records from biopsy surgery

Appendix 1. Records from biopsy surgery

12 mths, Jul/Aug 01							18 mths, Mar 02							24 mths, Jul/Aug 02								
ID	wt (kg)	pentothal 5% (mL)	surgeon *	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment	wt (kg)	pentothal 5% (mL)	surgeon	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment	wt (kg)	pentothal 5% (mL)	surgeon	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment	
1	23	8	mr	nr	nr	nr	much haemorrhage, constricted lumen of ileum	21	nr	nr	50	25	35	mild scarring at previous enterotomy site but no narrowing of lumen	23	8	mr	45	55	40	nr	
2	38	12	mr	nr	nr	nr	nr								37	15	cm	42	40	41	thickened ileum (had type 2 lesions on histo)	
4	27	8	rw	60	75	50	nr								31	20	cm	55	100	42	nr	
5	27	12	rc	55	50	45	nr								32	13	cc	35	55	30	cut through anastomosis	
6	20	10	mr	65	25	45	nr								28	10	rw	35	30	35	nr	
7	30	10	rc	55	45	37	nr	31	nr	rw	40	130	45	no sign of previous enterotomy site. Hematoma - 1cm diam formed at mesent border at enterotomy site - concerns re sequelae ??	29	13	cc	65	215	55	lot of adhesions around bowel. Very hard to get to LN due to adhesions Lot scar tissue in abdom wall	
8	24	12	rw	55	75	40	ileocaecal lymph node shelled out without suturing . Abdom wall closed 2 layers single continuous	26	13	mr	50	80	40	nr								ethanased with clincial OJD prior to surgery
10	22	14	mr	60	65	55	pentothal around jugular LHS - used RHS also to top up								27	13	cm	75	25	60	slight white at sight of previous sx	
11	20	13	rc	60	175	45	nr								31	10	rw	70	nr	68	nr	
12	26	10	rc	60	135	40	nr								32	10	mr	50	nr	50	spillage, broke down adhesion with slight hole in bowel repaired with 1 suture	
13	27	10	mr	55	60	50	nr								29	10	rw	60	75	70	nr	
14	28	10	rc	45	60	45	nr								35	14	mr	71	119	40	nr	
15	29	10	mr	60	45	50	slight gut spillage								25	15	cc	60	55	52	lots of adhesions- difficult to expose gut. Very thick ileum& caecum large lymph nodes (3c lesions on histo)	
16	25	8	rw	65	70	50	ileocaecal lymph node transected without suturing . Abdominal wall closed 2 layers single continuous								27	13	cm	43	105	98	nr	
17	30	10	mr	45	35	40	nr								30	13	cm	55	150	45	nr	
18	27	10	rc	nr	nr	nr	nr								39	15	cm	50	70	50	nr	
19	30	14	mr	nr	nr	nr	nr								30	13	rw	55	35	45	slight evidence of part surgery site line of minimal adhesion	
20	26	10	rc	50	105	40	nr								35	15	cs/cm	75	110	62	nr	
21	24	10	mr	55	55	50	nr								38	25	cc	60	170	40	nr	
22	30	12	rw	55	35	50	nr															
23	32	12	rc	nr	nr	nr	nr	34	13	mr	50	80	45	nr	35	15	cc	27	88	34	blood on ET tube	
24	20	10	mr	45	115	40	nr								25	17	cm	45	50	40	nr	
26	30	10	rc	55	65	45	nr								37	24	cj/cm	87	nr	70	nr	
27	32	12	mr	40	50	40	nr	34	15	rw	50	30	40	previous surgical site in ileum not noticeable	34	15	cm	70	50	60	nr	
28	26	10	mr	55	145	40	nr								37	14	cm	55	nr	50	nr	

Appendix 1. Records from biopsy surgery

12 mths, Jul/Aug 01							18 mths, Mar 02							24 mths, Jul/Aug 02							
ID	wt (kg)	pentothal 5% (mL)	surgeon *	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment	wt (kg)	pentothal 5% (mL)	surgeon	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment	wt (kg)	pentothal 5% (mL)	surgeon	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment
29	28	10	mr	60	100	50	nr								30	14	cm	60	65	55	nr
30	25	13	mr	60	105	35	nr	24	10	rw	45	90	35	no sign of previous surgery on ileum	29	16	mr	70	90	65	adhesions, slight spillage
31	29	10	mr	45	35	40	nr	36	12	mr	50	210	35	slight contamination. Haematoma in mesentery	33	15	mr	48	98	35	nr
32	30	10	rw	60	45	45	nr								30	12	cc	55	40	53	adhesion prevalent around post surgical site- needed to broken down to access ne sx site
33	24	12	mr	60	50	55	some extra bleeding								29	14	cm	42	43	39	nr
35	31	15	rc	60	40	60	leak at antimes. margin. Some reluctance to eat most day. Was bright & alert & ok when loaded to return to farm	35	nr	rw	50	55	40	scarring on serosal surface of ileum- no narrowing present	33	20	cj/cm	88	58	80	nr
36	26	10	mr	60	90	40	nr								29	13	cc	60	nr	45	nr
37	30	12	mr	50	65	45	nr								30	16	cm	54	74	41	nr
38	26	10	rc	57	62	53	nr								32	a lot	mr	57	186	37	nr
39	29	10	mr	45	65	40	nr								35	15	cc	50	35	37	nr
40	22	8	mr	nr	nr	nr	nr								28	14	cm	45	50	37	nr
41	23	12	rw	45	175	45	difficult intubation. There was apparent cording of lymphatic vessels associated with mesenteric border of terminal ileum .biopsy taken from such a region. (no lesions)								33	11	cm/cc	60	55	60	fibrin tags & adhesions evident around old surgical site
42	24	10	mr	60	140	50	flecks of faeces								29	18	cc	50	90	43	stressed post- op
43	31	10	mr	50	55	50	nr								34	15	cc	43	110	37	nr
44	23	10	rw	55	75	50	nr								29	17	cj/cc	105	nr	87	nr
45	24	10	rw	45	110	45	nr								30	14	cm	65	43	50	adhesions in musculature. Prominate fibre tract at past incision line in bowel.
46	30	14	rc	nr	nr	nr	haematoma distal to surgery site in (adjacent to) ileum- nasal discharge								30	15	cc	40	160	37	nr
47	24	10	rc	65	55	48	nr								26	15	cm	40	50	39	nr
49	26	10	rc	nr	nr	nr	nr								30	10	rc	40	30	35	nr
50	25	10	rw	55	65	45	nr	27	10	rw	55	70	45	2mm notch at antimes border of ileum from previous surgery- no narrowing of lumen. Terry reported "puffiness" & slight moisture at wound 10-3-02	25	18	mr	47	98	37	adhesions present & scar tissue around bowel sx site. Bleeding of caecal vessel when separated adhesions- clamped - ok
51	30	12	rc	55	95	45	nr	32	15	rw	60	90	40	scarring caecal surface of previous enterotomy site.	32	17	cm	15	65	50	nr
52	24	10	mr	60	60	55	muscular bleeding(small)								35	14	cm	60	25	46	nr

Appendix 1. Records from biopsy surgery

12 mths, Jul/Aug 01								18 mths, Mar 02								24 mths, Jul/Aug 02							
ID	wt (kg)	pentothal 5% (mL)	surgeon *	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment	wt (kg)	pentothal 5% (mL)	surgeon	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment	wt (kg)	pentothal 5% (mL)	surgeon	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment		
53	22	7.5	mr	nr	nr	nr	nr								32	19	cc	45	50	33	nr		
54	23	10	rw	15	60	45	nr	23	10	nr	40	nr	45	nr	26	12	cc	nr	nr	40	thickened bowel. Very large lymph node. (3c lesions, euthanased 4 mths later with OJD).		
55	27	12	rc	nr	nr	nr	difficult intubation - cyanotic on recovery had to mask with oxygen for approx 20 min														euthanased with clincial OJD prior to surgery		
56	22	8	rc	65	120	60	some gut spillage -narrowing lumen								22	10	rc	55	120	45	nr		
57	32	14	mr	nr	nr	nr	slight leakage faecal content between clamps . Took whole LN								35	14	mr	63	107	45	nr		
58	26	12	mr	50	90	45	nr	34	15	rw	40	65	35	no sign of previous sergery site in ileum	30	18	cc	45	nr	36	some adhesion to ileum		
59	25	10	mr	50	90	45	increased bleeding lymph node-bisal ligation								32	12	cc	43	40	40	nr		
60	25	12	rc	50	nr	50	nr								28	17	cc	55	20	40	nr		
61	30	10	rw	65	57	62	removed 1/2 ileocaecal lymph node but did not suture ab wall closed 2 keep suture contiuous								31	15	cc	53	82	45	nr		
62	25	10	rc	55	85	50	nr								35	16	cm	55	40	37	spillage		
63	29	10	mr	50	65	50	nr	32	15	mr	55	50	50	scar on serosal surface but no scarring of ileum. Terry reported "puffiness" slight moisture at wound 10-3-02	33	13	rw/cc	95	nr	59	slight frbrous tag joining last surgical site to mesentery		
64	30	12	rw	60	50	55	faecal contamination of surgical site& haemaerhage from lymph node								33	14	cc	45	80	35	nr		
65	24	9	rc	45	rc	35	nr	32	10	mr	60	30	40	nr	25	15	mr	55	55	50	nr		
66	27	12	rc	50	115	45	nr								30	14	cm	55	45	45	difficult		
67	22	12	rc	60	45	55	nr								26	8	rc	60	55	50	slight spillage		
68	24	10	mr	45	60	40	nr								28	13	mr	48	65	41	n		
69	29	10	rc	45	80	45	nr								30	10	cc	65	nr	60	spillage. Total resection-accidently		
70	28	10	rc	45	35	45	nr	27	12	mr	45	50	40	slight amount faecal matter in surgery site. Previous scaring very good , slight mesenteric adhesions.	31	17	cm	50	60	45	nr		
71	23	10	rc	55	70	55	nr								28	16	cc	55	80	55	past sx scar was apparent		
72	25	12	rc	nr	nr	nr	leakage faecal content between clamps	27	13	nr	40	85	30	very mild scarring at serosal surface ? no narrowing of lumen at previous surgery site	30	14	mr	55	65	52	nr		
73	21	10	rw	60	55	40	nr	27	10	mr	55	20	45	nr	28	13	mr	63	128	40	spillage		

Appendix 1. Records from biopsy surgery

12 mths, Jul/Aug 01							18 mths, Mar 02						24 mths, Jul/Aug 02									
ID	wt (kg)	pentothal 5% (mL)	surgeon *	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment	wt (kg)	pentothal 5% (mL)	surgeon	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment	wt (kg)	pentothal 5% (mL)	surgeon	duration anaesth (min)	time to stand (min)	duration surgery (min)	comment	
74	26	11	rc	50	70	45	nr								27	13	cm	58	30	48	thickened bowel (3b lesions, euth OJD 4 mth later)	
75	29	10	rw	55	65	45	some intestinal contents leaked from ileum onto drape, subcutaneous tissues. Additional lincocin onto sub cut tissues at closure.								35	20	cc	40	40	32	some spillage	
76	23	10	rc	45	60	40	nr	33	12	mr	50	65	45	nr								euthanased with clincial OJD prior to surgery
77	25	10	mr	42	45	42	nr	32	10	rc	50	45	50	Small leak when tested- sutured over- gut normal (no sign previous surg).	29	18	cm	45	120	40	nr	
78	28	14	rc	nr	nr	nr	some spillage from ileum - required extra sutures to seal leak.	28	10	mr	40	70	35	previous surgical site has scar visible on serosal surface but lumen is not narrowed	25	11	cm	45	nr	40	nr	
79	26	10	mr	60	75	50	some spillage								27	14	cm	65	65	50	nr	
80	30	14	rc	nr	nr	nr	nr								35	15	cm	48	55	46	lots of adhesions around previous surgical site	
81	27	10	mr	55	30	60	nr								31	10	mr	50	40	35	nr	
90	22	16	rc	50	85	50	nr								29	15	cc	50	55	29	nr	

* rc (R Churchill), rw (R Whittington), mr (M Richard), cc (C Corkhill), cm (C McConnel)

Indicates that gross lesions consistent with OJD seen at surgery, histo findings shown in parentheses after.

Appendix 2. Detailed test results at each sampling time for each sheep

Appendix 2a. Clinical assessments, sheep histories

Appendix 2b. Parachek Elisa

Appendix 2c. Agar gel immunodiffusion

Appendix 2d. Skin testing for delayed type hypersensitivity

Appendix 2e. IFN- γ

Appendix 2f. Tests for faecal excretion: Individual faecal culture, direct PCR

Appendix 2g. Histopathology results

Appendix 2h. Culture from tissues

Appendix 2a. Clinical assessments, sheep histories

ID	Sex	12 mths	18 mths	24 mths	30 mths	36 mths		Final OJD status	Age at death		
		Aug 01	Mar 02	Aug 02	Mar 03	wt	cond				Sep 03
		wt (kg)	wt (kg)	wt (kg)	wt (kg)	cond score		wt (kg)	cond score		
1	e	23	21	23	24	1		34	2.5	recovered	
2	w	38		37	38	2		46	2.5	mild OJD	
4	w	27		31	36	2.5		44	2.5	recovered	
5	e	27		32	33	2		40	3	mild OJD	
6	e	20		28	28	2		34	2	mild OJD	
7	e	30	31	29	32	2		40	2	recovered	
8		24	26							died OJD	24m clinical 1 month
10		22		27	26	1.5				died OJD	35m clinical < month
11	e	20		31	28	1.5		36	2.5	uninfected/n	
12	e	26		32	33	1.5		42	3	uninfected/r	
13		27		29						died OJD	29m clinical 1 week
14	e	28		35	33	1.5		40	2	mild OJD	
15	w	29		25	28	1.5		32	2	severe OJD	
16	e	25		27	29	2		38	2.5	mild OJD	
17	e	30		30	33	1.5		42	3	severe OJD	
18	e	27		39	37	2		42	2.5	uninfected/n	
19	e	30		30	33	2		38	3	mild OJD	
20	w	26		35	37	2		42	2.5	uninfected/n	
21	w	24		38	34	2		40	3	uninfected/n	
22		30								dead, mild OJD	24m died in paddock, no signs
23	w	32	34	35	33	1		34	2	mild OJD	
24	w	20		25	30	2		38	2.5	uninfected/n	
26	w	30		37	35	2		44	3	mild OJD	
27	w	32	34	34	38	2		44	3	mild OJD	
28	w	26		37	35	2.5		42	3	uninfected/n	
29		28		30						died OJD	29m clinical 1 week
30	w	25	24	29	29	1		38	2	recovered	
31	e	29	36	33	36	2.5		40	2.5	mild OJD	
32	w	30		30	36	2		44	2.5	uninfected/r	
33	e	24		29	34	2		40	3	uninfected/n	
35	w	31	35	33	39	2		46	3	mild OJD	
36	w	26		29	30	2		36	3	mild OJD	
37	e	30		30	32	2		40	3	mild OJD	
38	w	26		32	29	1	nec	32	2	uninfected/n	
39	w	29		35	36	2		44	2.5	mild OJD	
40	w	22		28	31	2		36	3	recovered	
41	w	23		33	36	2		46	3	mild OJD	
42	e	24		29	26	1.5		32	2	mild OJD	
43	w	31		34	36	2		46	2.5	mild OJD	
44	e	23		29	32	2		40	3	mild OJD	
45	e	24		30	34	2.5		38	3	uninfected/n	
46		30		30	29	1				died OJD	33m clinical < month
47	w	24		26	31	2		40	3	mild OJD	
49	e	26		30	31	2		36	2	severe OJD	
50	e	25	27	25	29	2		34	2.5	severe OJD	
51	e	30	32	32	34	2		42	3	recovered	
52	e	24		35	36	2.5		42	3	recovered	
53		22		32						dead, severe OJD	25m died in paddock, no signs
54		23	23	26 ^c						died OJD	28m clinical 6 mths
55		27								died OJD	24m clinical 1 mth
56	e	22		22	30	2		32	2.5	mild OJD	
57	w	32		35	38	2.5		44	3	recovered	
58		26	34	30	32	1.5				died OJD	34m clinical < month
59	e	25		32	31	2		36	2.5	uninfected/n	
60	w	25		28	33	2		30	2	severe OJD	
61	w	30		31	36	2		42	2.5	mild OJD	
62	w	25		35	40	2		50	3	mild OJD	
63	e	29	32	33	38	2		42	2.5	mild OJD	
64	e	30		33	35	2		40	2.5	recovered	
65	e	24	32	25	29	1.5		38	2.5	mild OJD	
66	w	27		30	34	1.5		42	3	mild OJD	
67	e	22		26	27	2		32	3	mild OJD	
68		24		28						dead, uninfected/	25m died in paddock, no signs
69	e	29		30	35	2		40	2.5	uninfected/r	

Appendix 2a. Clinical assessments, sheep histories

ID	Sex	12 mths	18 mths	24 mths	30 mths	36 mths		Final OJD status	Age at death
		Aug 01	Mar 02	Aug 02	Mar 03	wt	cond		
		wt (kg)	wt (kg)	wt (kg)	wt (kg)	cond score	wt (kg)	cond score	
70	e	28	27	31	35	2	38	3	mild OJD
71	w	23		28	28	1.5	40	3	severe OJD
72	e	25	27	30	33	2	38	2.5	recovered
73	w	21	27	28	30	2	36	2.5	recovered
74		26		27					died OJD 28m clinical 1 month
75	e	29		35	36	2.5	40	3	uninfected/n
76		23	33						died OJD 24m clinical < month
77	w	25	32	29	34	2.5	42	3	mild OJD
78		28	28	25					died OJD 29m clinical < month
79	e	26		27	33	2	40	3	mild OJD
80	w	30		35	35	2	44	3	mild OJD
81	e	27		31	33	2	38	2.5	uninfected/r
90	w	22		29	32	2	40	3	uninfected/r

^c Clinical signs of OJD

Appendix 2b. Parachek Elisa results, details

ID	18 months		24 months		30 months		36 months	
	Mar 02		Sep 02		Mar 03		Sep 03	
	OD450nm	el-18	OD450nm	el-24	OD450nm	el-30	OD450nm	EL-36
1	0.072	-	0.517	+	0.086	-	0.196	-
2	0.070	-	0.093	-	0.056	-	0.062	-
4	0.071	-	0.112	-	0.067	-	0.092	-
5	0.066	-	0.201	-	0.283	+	0.105	-
6	0.129	-	0.186	-	0.066	-	0.120	-
7	0.080	-	0.119	-	0.062	-	0.113	-
8	1.634	+						
10	0.463	+	0.770	+	0.952	+	0.765	+
11	0.069	-	0.105	-	0.065	-	0.084	-
12	0.130	-	0.096	-	0.098	-	0.104	-
13	0.096	-	0.226	-	0.285	+		
14	0.104	-	0.204	-	0.067	-	0.144	-
15	0.112	-	0.215	-	0.111	-	0.143	-
16	0.050	-	0.117	-	0.058	-	0.064	-
17	0.080	-	0.089	-	0.069	-	0.286	+
18	0.062	-	0.105	-	0.055	-	0.066	-
19	0.066	-	0.112	-	0.060	-	0.075	-
20	0.088	-	0.174	-	0.056	-	0.066	-
21	0.068	-	0.138	-	0.063	-	0.080	-
22	0.126	-	0.134	-				
23	0.056	-	0.082	-	0.131	-	0.214	-
24	0.069	-	0.077	-	0.056	-	0.060	-
26	0.225	-	0.251	-	0.103	-	0.098	-
27	0.087	-	0.106	-	0.075	-	0.877	+
28	0.055	-	0.062	-	0.062	-	0.050	-
29	0.176	-	0.272	+	2.786	+		
30	0.279	+	0.285	+	0.106	-	0.180	-
31	0.102	-	0.124	-	0.053	-	0.082	-
32	0.084	-	0.120	-	0.055	-	0.059	-
33	0.052	-	0.079	-	0.052	-	0.058	-
35	0.089	-	0.151	-	0.084	-	0.077	-
36	0.054	-	0.100	-	0.056	-	0.068	-
37	0.076	-	0.175	-	0.050	-	0.068	-
38	0.072	-	0.089	-	0.062	-	0.062	-
39	0.059	-	0.095	-	0.098	-	0.068	-
40	0.067	-	0.089	-	0.051	-	0.057	-
41	0.058	-	0.072	-	0.045	-	0.057	-
42	0.074	-	0.092	-	0.067	-	0.092	-
43	0.068	-	0.175	-	0.060	-	0.066	-
44	0.072	-	0.119	-	0.068	-	0.117	-
45	0.068	-	0.127	-	0.059	-	0.089	-
46	0.064	-	0.156	-	1.364	+	0.531	+
47	0.066	-	0.187	-	0.095	-	0.204	-
49	0.053	-	0.097	-	0.076	-	0.301	+
50	0.130	-	0.750	+	0.087	-	0.242	-
51	0.068	-	0.089	-	0.060	-	0.061	-
52	0.055	-	0.104	-	0.062	-	0.063	-
53	0.063	-	0.067	-				
54	2.592	+	3.183	+				
55	0.066	-	0.409	+				
56	0.058	-	0.100	-	0.062	-	0.090	-

Appendix 2b. Parachek Elisa results, details

ID	18 months		24 months		30 months		36 months	
	Mar 02		Sep 02		Mar 03		Sep 03	
	OD450nm	el-18	OD450nm	el-24	OD450nm	el-30	OD450nm	EL-36
57	0.051	-	0.074	-	0.059	-	0.078	-
58	0.058	-	0.099	-	0.063	-	0.351	+
59	0.062	-	0.081	-	0.059	-	0.052	-
60	0.076	-	0.142	-	0.133	-	0.251	-
61	0.048	-	0.082	-	0.054	-	0.059	-
62	0.059	-	0.109	-	0.056	-	0.058	-
63	1.782	+	0.908	+	0.738	+	0.256	+
64	0.081	-	0.105	-	0.050	-	0.079	-
65	0.680	+	0.734	+	0.381	+	0.224	-
66	0.064	-	0.105	-	0.053	-	0.066	-
67	0.076	-	0.142	-	0.190	-	0.112	-
68	0.083	-	0.085	-				
69	0.128	-	0.107	-	0.076	-	0.089	-
70	0.063	-	0.088	-	0.057	-	0.064	-
71	0.169	-	0.153	-	0.059	-	0.083	-
72	0.226	-	0.382	+	0.251	-	0.141	-
73	0.083	-	0.062	-	0.054	-	0.082	-
74	0.094	-	0.154	-				
75	0.061	-	0.124	-	0.055	-	0.187	-
76	0.082	-	0.313	+				
77	0.090	-	0.130	-	0.059	-	0.064	-
78	0.065	-	0.093	-	0.200	-		
79	0.065	-	0.118	-	0.054	-	0.074	-
80	0.056	-	0.092	-	0.054	-	0.066	-
81	0.077	-	0.130	-	0.052	-	0.062	-
90	0.125	-	0.086	-	0.068	-	0.059	-

Cut-off points were: 18m, 0.252; 24m, 0.256; 30m, 0.255; 36m, 0.251

Appendix 2c. Agar gel immunodiffusion results (gel test)

Sheep ID	9	12	18	24	30	36	Date of necropsy
	Jun-01	Sep-01	Mar-02	Aug-02	Mar-03	Sep-03	
1	-	-	-	-	-	-	
2	-	-	-	-	-	-	
4	-	-	-	-	-	-	
5	-	-	-	-	-	-	
6	-	-	-	-	-	-	
7	-	-	-	-	-	-	
8	-	-	-	+++	-	-	aug-02
10	-	-	-	+	++	++	aug-03
11	-	-	-	-	-	-	
12	-	-	-	-	-	-	
13	-	-	-	-	+++	-	feb-03
14	-	-	-	-	-	-	
15	-	-	-	-	-	-	
16	-	-	-	-	-	-	
17	-	-	-	-	-	-	
18	-	-	-	-	-	-	
19	-	-	-	-	-	-	
20	-	-	-	-	-	-	
21	-	-	-	-	-	-	
22	-	-	-	-	-	-	died in paddoc
23	-	-	-	-	-	-	
24	-	-	-	-	-	-	
26	-	-	-	-	-	-	
27	-	-	-	-	-	-	
28	-	-	-	-	-	-	
29	-	-	-	-	+++	-	feb-03
30	-	-	+	-	+	-	
31	-	-	-	-	-	-	
32	-	-	-	-	-	-	
33	-	-	-	-	-	-	
35	-	-	-	-	-	-	
36	-	-	-	-	-	-	
37	-	-	-	-	-	-	
38	-	-	-	-	-	-	
39	-	-	-	-	-	-	
40	-	-	-	-	-	-	
41	-	-	-	-	-	-	
42	-	-	-	-	-	-	
43	-	-	-	-	-	-	
44	-	-	-	-	-	-	
45	-	-	-	-	-	-	
46	-	-	-	-	-	inc	may-03
47	-	-	-	-	-	-	
49	-	-	-	-	-	+	
50	-	-	-	-	+	-	
51	-	-	-	-	-	-	
52	-	-	-	-	-	-	
53	-	-	-	-	-	-	died in paddoc
54	-	-	++	+++	++	-	jan-03
55	-	-	-	+++	-	-	aug-02
56	-	-	-	-	-	-	
57	-	-	-	-	-	-	
58	-	-	-	-	+	+++	july-03
59	-	-	-	-	-	-	
60	-	-	-	-	-	+	
61	-	-	-	-	-	-	
62	-	-	-	-	-	-	
63	-	-	-	-	-	-	

Appendix 2c. Agar gel immunodiffusion results (gel test)

Sheep ID	9	12	18	24	30	36	Date of necropsy
	Jun-01	Sep-01	Mar-02	Aug-02	Mar-03	Sep-03	
64	-	-	-	-	-	-	
65	-	-	-	-	-	-	
66	-	-	-	-	-	-	
67	-	-	-	-	-	-	
68	-	-	-	-	-	-	died in paddoc
69	-	-	-	-	-	-	
70	-	-	-	-	-	-	
71	-	-	-	-	-	-	
72	-	-	-	-	-	-	
73	-	-	-	-	-	-	
74	-	-	-	-	+++	-	jan-03
75	-	-	-	-	-	-	
76	-	-	-	-	-	-	aug-02
77	-	-	-	-	-	-	
78	-	-	-	-	+++	-	feb-03
79	-	-	-	-	-	-	
80	-	-	-	-	-	-	
81	-	-	-	-	-	-	
90	-	-	-	-	-	-	

These samples collected at necropsy examination.

Appendix 2c. Skin testing for delayed type hypersensitivity

Sheep ID	24 months				30 months				36 months			
	0 h	72 h	Increase	dth-24	0 h	72 h	Increase	dth-30	0 h	72 h	Increase	dth-36
1	0.5	4.9	4.4	+	0.4	4	3.6	-	0.9	2.1	1.2	-
2	0.8	5.4	4.6	+	0.5	1.6	1.1	-	0.5	2.2	1.7	-
4	0.5	3.1	2.6	-	0.5	2	1.5	-	0.4	1.9	1.5	-
5	0.4	4	3.6	-	0.7	1.5	0.8	-	0.7	2	1.3	-
6	0.8	3.9	3.1	-	0.6	3.8	3.2	-	0.4	2	1.6	-
7	0.6	8.1	7.5	+	0.5	4.6	4.1	+	0.5	1.4	0.9	-
8												
10	0.5	6	5.5	+	0.6	3	2.4	-				
11	0.4	3.7	3.3	-	0.5	2.2	1.7	-	0.4	2	1.6	-
12	0.4	4	3.6	-	0.5	2.4	1.9	-	0.4	2.5	2.1	-
13	0.5	4.4	3.9	-	-							
14	0.9	1.7	0.8	-	0.6	1.7	1.1	-	0.8	1.4	0.6	-
15	0.4	7	6.6	+	0.4	2.3	1.9	-	0.4	3.1	2.7	-
16	0.5	3	2.5	-	0.5	2.1	1.6	-	0.4	2.7	2.3	-
17	0.6	2.5	1.9	-	0.8	1.2	0.4	-	0.5	5	4.5	+
18	0.5	3.8	3.3	-	0.5	2.4	1.9	-	0.5	2.1	1.6	-
19	0.5	0.5	0	-	0.8	5.5	4.7	+	1	4	3.0	-
20	0.6	1.4	0.8	-	0.5	1.6	1.1	-	0.8	1.9	1.1	-
21	0.6	1.3	0.7	-	0.5	3.4	2.9	-	0.5	3.5	3.0	-
22	0.6	4.2	3.6	-								
23	0.6	1.5	0.9	-	0.5	8.5	8	+	0.5	2.6	2.1	-
24	0.5	3	2.5	-	0.5	2	1.5	-	0.5	1.8	1.3	-
26	0.9	3	2.1	-	0.4	1.4	1	-	0.8	1.4	0.6	-
27	0.8	8.5	7.7	+	0.5	10	9.5	+	0.4	4	3.6	-
28	0.4	1.3	0.9	-	0.6	2.1	1.5	-	0.4	2	1.6	-
29	0.5	7	6.5	+	-							
30	0.7	9	8.3	+	0.5	4	3.5	-	0.4	3	2.6	-
31	0.9	9	8.1	+	0.5	3.4	2.9	-	1	3	2.0	-
32	0.9	6.1	5.2	+	0.8	5.6	4.8	+	0.6	4.5	3.9	-
33	0.5	2	1.5	-	0.5	1.3	0.8	-	0.4	1.9	1.5	-
35	0.4	8.2	7.8	+	0.5	4.5	4	-	0.5	3	2.5	-
36	0.5	2	1.5	-	0.6	2	1.4	-	0.4	2.5	2.1	-
37	0.5	1.4	0.9	-	0.5	1.5	1	-	0.5	2.1	1.6	-
38	0.6	2.1	1.5	-	0.4	2.5	2.1	-	0.6	1.3	0.7	-
39	0.7	2.7	2	-	0.6	3.2	2.6	-	0.8	3.4	2.6	-
40	0.5	3.1	2.6	-	0.5	2	1.5	-	0.7	2.4	1.7	-
41	0.4	2.6	2.2	-	0.6	2	1.4	-	1	2.3	1.3	-
42	0.5	5.1	4.6	+	0.5	4	3.5	-	0.5	3.5	3.0	-
43	0.5	3	2.5	-	0.6	2	1.4	-	0.9	2.7	1.8	-
44	0.5	3	2.5	-	0.5	4.4	3.9	-	0.7	2.3	1.6	-
45	0.6	1.6	1	-	0.6	2.4	1.8	-	0.4	2	1.6	-
46	0.5	5.4	4.9	+	0.6	9.4	8.8	+				
47	0.4	2.1	1.7	-	0.5	1.9	1.4	-	0.4	1.4	1.0	-
49	0.5	5.4	4.9	+	0.6	3.5	2.9	-	0.4	3.1	2.7	-
50	0.4	7	6.6	+	0.5	8.9	8.4	+	0.8	5	4.2	+
51	0.5	3.4	2.9	-	0.6	3.6	3	-	0.5	3.1	2.6	-
52	1	2.8	1.8	-	0.5	2	1.5	-	0.5	2.2	1.7	-
53	0.6	5.4	4.8	+								
54	0.5	3.1	2.6	-								
55	0.5	4	3.5	-								
56	0.5	1.5	1	-	0.5	2.4	1.9	-	0.4	3	2.6	-
57	0.9	5	4.1	+	0.8	4	3.2	-	0.4	3.1	2.7	-
58	0.8	7.1	6.3	+	0.8	4.7	3.9	-				
59	0.7	2	1.3	-	0.5	2.3	1.8	-	0.9	2.5	1.6	-
60	0.5	3	2.5	-	0.6	2	1.4	-	0.4	1.5	1.1	-

Appendix 2c. Skin testing for delayed type hypersensitivity

Sheep ID	24 months				30 months				36 months			
	0 h	72 h	Increase	dth-24	0 h	72 h	Increase	dth-30	0 h	72 h	Increase	dth-36
61	0.6	4	3.4	-	0.8	2.5	1.7	-	0.4	2.1	1.7	-
62	0.6	4	3.4	-	0.5	2	1.5	-	0.8	2	1.2	-
63	0.5	7	6.5	+	0.6	3.4	2.8	-	0.8	2.7	1.9	-
64	0.8	2.1	1.3	-	0.6	2.1	1.5	-	0.4	2.1	1.7	-
65	1	8.4	7.4	+	0.5	6.4	5.9	+	0.8	3.8	3.0	-
66	0.5	1.7	1.2	-	0.5	1.5	1	-	0.4	1.6	1.2	-
67	0.5	4	3.5	-	0.8	2.2	1.4	-	0.5	2.1	1.6	-
68	0.5	4	3.5	-								
69	0.5	4.4	3.9	-	0.6	2.2	1.6	-	0.8	2.1	1.3	-
70	0.6	3.1	2.5	-	0.5	2	1.5	-	0.4	2.4	2.0	-
71	0.9	1.9	1	-	0.5	2.4	1.9	-	0.8	3.2	2.4	-
72	0.5	6	5.5	+	0.8	3.8	3	-	0.5	4	3.5	-
73	0.4	2.1	1.7	-	0.9	2.1	1.2	-	0.7	1.1	0.4	-
74	0.5	5	4.5	+								
75	0.7	3	2.3	-	0.5	3.4	2.9	-	0.5	3	2.5	-
76	0.7	3	2.3	-								
77	0.5	2.4	1.9	-	0.4	2.4	2	-	0.5	2.1	1.6	-
78	0.5	6	5.5	+	-							
79	0.6	1	0.4	-	0.7	2.7	2	-	0.9	3	2.1	-
80	0.6	1.4	0.8	-	0.5	2	1.5	-	0.5	3.2	2.7	-
81	0.6	5.8	5.2	+	0.4	3	2.6	-	1	4.1	3.1	-
90	0.5	2	1.5	-	0.5	2.7	2.2	-	0.9	2.1	1.2	-

Gamma interferon results - 9 months, Jun 2001

SheepID	Optical densities						Result*
	av	jo	c	a-c	j-c	j-a	
1	0.052	0.05	0.048	0.004	0.00	0.00	-
2	0.048	0.042	0.037	0.011	0.01	-0.01	-
4	0.046	0.021	0.033	0.013	-0.01	-0.03	-
5	0.05	0.031	0.03	0.02	0.00	-0.02	-
6	0.051	0.031	0.032	0.019	0.00	-0.02	-
7	0.064	0.097	0.036	0.028	0.06	0.03	-
8	0.044	0.026	0.033	0.011	-0.01	-0.02	-
10	0.044	0.033	0.033	0.011	0.00	-0.01	-
11	0.042	0.032	0.03	0.012	0.00	-0.01	-
12	0.047	0.044	0.037	0.01	0.01	0.00	-
13	0.032	0.036	0.042	-0.01	-0.01	0.00	-
14	0.03	0.037	0.028	0.002	0.01	0.01	-
15	0.051	0.056	0.053	-0.002	0.00	0.01	-
16	0.036	0.039	0.046	-0.01	-0.01	0.00	-
17	0.043	0.042	0.044	-0.001	0.00	0.00	-
18	0.049	0.049	0.043	0.006	0.01	0.00	-
19	0.054	0.046	0.044	0.01	0.00	-0.01	-
20	0.058	0.058	0.046	0.012	0.01	0.00	-
21	0.047	0.045	0.046	0.001	0.00	0.00	-
22	0.057	0.052	0.049	0.008	0.00	-0.01	-
23	0.047	0.041	0.044	0.003	0.00	-0.01	-
24	0.046	0.04	0.045	0.001	-0.01	-0.01	-
26	0.051	0.044	0.053	-0.002	-0.01	-0.01	-
27	0.09	0.17	0.046	0.044	0.12	0.08	+
28	0.06	0.051	0.051	0.009	0.00	-0.01	-
29	0.075	0.133	0.044	0.031	0.09	0.06	+
30	0.058	0.057	0.053	0.005	0.00	0.00	-
31	0.049	0.046	0.046	0.003	0.00	0.00	-
32	0.045	0.064	0.045	0	0.02	0.02	-
33	0.05	0.053	0.059	-0.009	-0.01	0.00	-
35	0.034	0.042	0.043	-0.009	0.00	0.01	-
36	0.047	0.041	0.053	-0.006	-0.01	-0.01	-
37	0.052	0.051	0.035	0.017	0.02	0.00	-
38	0.054	0.049	0.032	0.022	0.02	-0.01	-
39	0.043	0.039	0.027	0.016	0.01	0.00	-
40	0.049	0.044	0.026	0.023	0.02	-0.01	-
41	0.046	0.042	0.029	0.017	0.01	0.00	-
42	no sample received						-
43	0.04	0.039	0.031	0.009	0.01	0.00	-
44	0.046	0.04	0.036	0.01	0.00	-0.01	-
45	0.05	0.045	0.042	0.008	0.00	-0.01	-
46	0.08	0.073	0.07	0.01	0.00	-0.01	-
47	0.054	0.062	0.041	0.013	0.02	0.01	-
49	0.059	0.064	0.053	0.006	0.01	0.01	-
50	0.074	0.125	0.051	0.023	0.07	0.05	+
51	0.052	0.054	0.047	0.005	0.01	0.00	-
52	0.055	0.049	0.041	0.014	0.01	-0.01	-
53	0.049	0.051	0.038	0.011	0.01	0.00	-
54	0.056	0.062	0.049	0.007	0.01	0.01	-
55	0.052	0.055	0.044	0.008	0.01	0.00	-
56	0.048	0.039	0.032	0.016	0.01	-0.01	-

Gamma interferon results - 9 months, Jun 2001

SheepID	Optical densities						Result*
	av	jo	c	a-c	j-c	j-a	
57	0.063	0.052	0.037	0.026	0.02	-0.01	-
58	0.05	0.035	0.021	0.029	0.01	-0.02	-
59	0.058	0.037	0.025	0.033	0.01	-0.02	-
60	0.055	0.031	0.04	0.015	-0.01	-0.02	-
61	0.041	0.043	0.042	-0.001	0.00	0.00	-
62	0.047	0.047	0.046	0.001	0.00	0.00	-
63	0.069	0.175	0.052	0.017	0.12	0.11	+
64	0.047	0.049	0.049	-0.002	0.00	0.00	-
65	0.054	0.064	0.054	0	0.01	0.01	-
66	0.046	0.044	0.045	0.001	0.00	0.00	-
67	0.056	0.06	0.059	-0.003	0.00	0.00	-
68	0.056	0.057	0.055	0.001	0.00	0.00	-
69	0.053	0.046	0.045	0.008	0.00	-0.01	-
70	0.05	0.049	0.048	0.002	0.00	0.00	-
71	0.041	0.039	0.042	-0.001	0.00	0.00	-
72	0.078	0.192	0.057	0.021	0.14	0.11	+
73	0.046	0.049	0.041	0.005	0.01	0.00	-
74	0.046	0.045	0.04	0.006	0.01	0.00	-
75	0.042	0.037	0.034	0.008	0.00	-0.01	-
76	0.348	1.046	0.041	0.307	1.01	0.70	+
77	0.055	0.063	0.051	0.004	0.01	0.01	-
78	0.041	0.036	0.029	0.012	0.01	-0.01	-
79	0.072	0.066	0.062	0.01	0.00	-0.01	-
80	0.045	0.041	0.036	0.009	0.01	0.00	-
81	0.045	0.026	0.036	0.009	-0.01	-0.02	-
90	0.052	0.042	0.039	0.013	0.00	-0.01	-

* Positive if OD(Johnin) - OD(Avian) > 0.05

Gamma interferon results - 12 months, Sep 2001

SheepID	Optical densities			a-c	j-c	j-a	Result*
	av	jo	c				
1	0.044	0.050	0.034	0.010	0.017	0.007	-
2	0.041	0.043	0.045	-0.004	-0.003	0.002	-
4	0.034	0.030	0.035	-0.002	-0.005	-0.004	-
5	0.058	0.063	0.057	0.001	0.006	0.005	-
6	0.044	0.045	0.040	0.004	0.006	0.002	-
7	0.036	0.052	0.028	0.008	0.024	0.016	-
8	0.054	0.046	0.037	0.017	0.009	-0.008	-
10	0.045	0.048	0.048	-0.004	-0.001	0.003	-
11	0.042	0.050	0.054	-0.012	-0.005	0.008	-
12	0.018	0.020	0.030	-0.012	-0.011	0.002	-
13	0.030	0.030	0.037	-0.007	-0.008	-0.001	-
14	0.051	0.042	0.037	0.015	0.006	-0.009	-
15	0.046	0.033	0.030	0.017	0.004	-0.013	-
16	0.046	0.047	0.052	-0.006	-0.005	0.001	-
17	0.021	0.010	0.016	0.005	-0.006	-0.011	-
18	0.048	0.043	0.035	0.013	0.008	-0.005	-
19	0.033	0.027	0.037	-0.004	-0.010	-0.006	-
20	0.033	0.015	0.019	0.014	-0.004	-0.018	-
21	0.020	0.013	0.024	-0.004	-0.012	-0.008	-
22	0.063	0.046	0.039	0.025	0.008	-0.017	-
23	0.060	0.043	0.031	0.030	0.013	-0.017	-
24	0.055	0.038	0.039	0.016	-0.001	-0.017	-
26	0.058	0.048	0.036	0.022	0.012	-0.010	-
27	0.040	0.050	0.022	0.018	0.029	0.011	-
28	0.074	0.069	0.075	-0.001	-0.006	-0.005	-
29	0.048	0.056	0.040	0.008	0.016	0.009	-
30	0.042	0.047	0.034	0.008	0.013	0.005	-
31	0.052	0.041	0.027	0.025	0.014	-0.012	-
32	0.034	0.029	0.037	-0.003	-0.008	-0.005	-
33	0.059	0.047	0.035	0.024	0.012	-0.013	-
35	0.030	0.029	0.029	0.002	0.001	-0.001	-
36	0.024	0.033	0.042	-0.018	-0.009	0.009	-
37	0.050	0.044	0.040	0.010	0.004	-0.007	-
38	0.032	0.027	0.034	-0.003	-0.007	-0.005	-
39	0.041	0.038	0.053	-0.012	-0.015	-0.003	-
40	0.058	0.042	0.025	0.033	0.017	-0.016	-
41	0.091	0.094	0.084	0.007	0.010	0.003	-
42	0.045	0.039	0.040	0.005	-0.002	-0.007	-
43	0.036	0.014	0.026	0.010	-0.012	-0.022	-
44	0.048	0.041	0.036	0.012	0.005	-0.007	-
45	0.048	0.046	0.038	0.010	0.007	-0.003	-
46	0.069	0.055	0.045	0.025	0.011	-0.014	-
47	0.052	0.046	0.047	0.005	-0.002	-0.006	-
49	0.056	0.039	0.029	0.027	0.010	-0.017	-
50	0.061	0.056	0.040	0.021	0.017	-0.005	-
51	0.029	0.021	0.016	0.014	0.006	-0.008	-
52	0.055	0.037	0.033	0.022	0.004	-0.018	-
53	0.052	0.038	0.036	0.016	0.003	-0.014	-
54	0.051	0.040	0.038	0.013	0.002	-0.011	-
55	0.026	0.032	0.040	-0.014	-0.008	0.006	-
56	0.052	0.042	0.035	0.017	0.006	-0.010	-

Gamma interferon results - 12 months, Sep 2001

SheepID	Optical densities			a-c	j-c	j-a	Result*
	av	jo	c				
57	0.044	0.032	0.044	-0.001	-0.012	-0.012	-
58	0.026	0.026	0.034	-0.008	-0.008	0.000	-
59	0.035	0.039	0.038	-0.004	0.001	0.004	-
60	0.028	0.032	0.041	-0.013	-0.010	0.004	-
61	0.037	0.032	0.034	0.003	-0.002	-0.005	-
62	0.053	0.036	0.018	0.035	0.018	-0.017	-
63	0.065	0.052	0.036	0.029	0.016	-0.013	-
64	0.024	0.014	0.028	-0.004	-0.014	-0.011	-
65	0.061	0.049	0.040	0.021	0.010	-0.012	-
66	0.046	0.045	0.051	-0.005	-0.006	-0.001	-
67	0.040	0.035	0.036	0.004	-0.001	-0.005	-
68	0.032	0.034	0.036	-0.005	-0.003	0.002	-
69	0.020	0.024	0.036	-0.016	-0.012	0.004	-
70	0.042	0.037	0.037	0.005	0.000	-0.005	-
71	0.051	0.038	0.030	0.021	0.008	-0.013	-
72	0.039	0.072	0.033	0.006	0.040	0.034	-
73	0.016	0.010	0.025	-0.009	-0.015	-0.006	-
74	0.046	0.043	0.035	0.012	0.008	-0.004	-
75	0.040	0.033	0.036	0.004	-0.003	-0.007	-
76	0.085	0.217	0.061	0.025	0.156	0.132	+
77	0.028	0.021	0.037	-0.009	-0.016	-0.007	-
78	0.039	0.039	0.032	0.007	0.007	0.000	-
79	0.049	0.040	0.034	0.015	0.006	-0.010	-
80	0.034	0.028	0.026	0.008	0.002	-0.006	-
81	0.037	0.033	0.031	0.006	0.002	-0.004	-
90	0.021	0.027	0.035	-0.015	-0.008	0.007	-

* Positive if OD(Johnin) - OD(Avian) > 0.05

Gamma interferon results - 18 months, Feb 2002

SheepID	Optical densities			a-c	j-c	j-a	Result*
	av	jo	c				
1	0.076	0.101	0.068	0.008	0.034	0.025	-
2	0.126	0.142	0.138	-0.013	0.004	0.017	-
4	0.078	0.078	0.094	-0.016	-0.016	-0.001	-
5	0.090	0.075	0.073	0.017	0.002	-0.015	-
6	0.058	0.069	0.054	0.005	0.016	0.011	-
7	0.147	0.183	0.110	0.037	0.073	0.036	-
8	0.076	0.161	0.050	0.026	0.112	0.086	+
10	0.404	0.731	0.079	0.326	0.652	0.327	+
11	0.069	0.084	0.068	0.001	0.016	0.015	-
12	0.059	0.060	0.044	0.016	0.017	0.001	-
13	0.040	0.083	0.063	-0.023	0.020	0.043	-
14	0.078	0.096	0.079	-0.001	0.017	0.018	-
15	0.078	0.092	0.090	-0.012	0.002	0.014	-
16	0.049	0.040	0.059	-0.010	-0.019	-0.009	-
17	0.074	0.099	0.113	-0.040	-0.015	0.025	-
18	0.036	0.029	0.036	0.001	-0.007	-0.007	-
19	0.055	0.042	0.051	0.004	-0.009	-0.013	-
20	0.058	0.038	0.036	0.022	0.002	-0.020	-
21	0.072	0.071	0.049	0.023	0.022	-0.001	-
22	0.113	0.235	0.098	0.016	0.138	0.122	+
23	0.047	0.063	0.072	-0.025	-0.009	0.016	-
24	0.095	0.100	0.104	-0.008	-0.003	0.005	-
26	0.058	0.078	0.067	-0.009	0.011	0.020	-
27	0.152	0.262	0.048	0.105	0.214	0.110	+
28	0.049	0.029	0.041	0.009	-0.012	-0.021	-
29	0.165	0.374	0.040	0.126	0.334	0.209	+
30	0.121	0.205	0.110	0.012	0.096	0.084	+
31	0.042	0.048	0.032	0.010	0.016	0.006	-
32	0.056	0.053	0.059	-0.004	-0.006	-0.003	-
33	0.058	0.048	0.043	0.015	0.005	-0.010	-
35	0.111	0.129	0.091	0.021	0.039	0.018	-
36	0.051	0.028	0.044	0.007	-0.016	-0.023	-
37	0.082	0.069	0.111	-0.029	-0.042	-0.013	-
38	0.058	0.053	0.035	0.024	0.018	-0.006	-
39	0.052	0.038	0.037	0.015	0.001	-0.014	-
40	0.048	0.034	0.029	0.019	0.005	-0.014	-
41	0.102	0.113	0.135	-0.033	-0.022	0.011	-
42	0.109	0.125	0.120	-0.012	0.005	0.016	-
43	0.066	0.047	0.039	0.027	0.008	-0.020	-
44	0.065	0.044	0.051	0.014	-0.007	-0.021	-
45	0.041	0.046	0.030	0.012	0.017	0.005	-
46	0.103	0.102	0.103	-0.001	-0.002	-0.001	-
47	0.031	0.026	0.025	0.006	0.001	-0.005	-
49	0.072	0.070	0.050	0.022	0.020	-0.002	-
50	0.223	0.394	0.135	0.088	0.259	0.171	+
51	0.123	0.157	0.067	0.056	0.090	0.034	-
52	0.056	0.058	0.057	-0.001	0.001	0.002	-
53	0.063	0.049	0.039	0.024	0.011	-0.014	-
54	0.168	0.340	0.095	0.073	0.245	0.172	+
55	0.078	0.096	0.045	0.033	0.051	0.018	-
56	0.061	0.066	0.039	0.022	0.027	0.005	-

Gamma interferon results - 18 months, Feb 2002

SheepID	Optical densities			a-c	j-c	j-a	Result*
	av	jo	c				
57	0.066	0.117	0.084	-0.019	0.033	0.051	-
58	0.101	0.116	0.032	0.069	0.084	0.016	-
59	0.045	0.032	0.055	-0.010	-0.023	-0.013	-
60	0.050	0.043	0.031	0.020	0.012	-0.008	-
61	0.062	0.084	0.053	0.009	0.031	0.022	-
62	0.060	0.079	0.054	0.006	0.025	0.019	-
63	0.104	0.124	0.083	0.021	0.041	0.020	-
64	0.047	0.034	0.036	0.012	-0.002	-0.013	-
65	0.076	0.099	0.047	0.029	0.052	0.023	-
66	0.105	0.114	0.077	0.029	0.037	0.009	-
67	0.137	0.152	0.092	0.045	0.060	0.016	-
68	0.079	0.099	0.088	-0.008	0.012	0.020	-
69	0.065	0.061	0.054	0.011	0.008	-0.004	-
70	0.241	0.247	0.183	0.059	0.065	0.006	-
71	0.110	0.107	0.099	0.011	0.008	-0.003	-
72	0.082	0.107	0.043	0.040	0.064	0.025	-
73	0.056	0.041	0.059	-0.003	-0.018	-0.015	-
74	0.067	0.101	0.085	-0.018	0.016	0.034	-
75	0.035	0.033	0.026	0.009	0.007	-0.002	-
76	0.169	0.413	0.084	0.085	0.329	0.245	+
77	0.121	0.184	0.188	-0.067	-0.005	0.063	-
78	0.345	0.953	0.090	0.255	0.863	0.608	+
79	0.049	0.054	0.057	-0.007	-0.003	0.005	-
80	0.102	0.114	0.134	-0.032	-0.020	0.012	-
81	0.146	0.129	0.121	0.025	0.008	-0.017	-
90	0.080	0.099	0.078	0.002	0.022	0.020	-

* Positive if OD(Johnin) - OD(Avian) > 0.05

Gamma interferon results - 24 months, Aug 2002

SheepID	Optical densities			a-c	j-c	j-a	Result*
	av	jo	c				
1	0.223	0.280	0.095	0.128	0.186	0.058	+
2	0.092	0.131	0.085	0.006	0.046	0.040	-
4	0.072	0.072	0.045	0.027	0.027	0.000	-
5	0.119	0.115	0.073	0.046	0.042	-0.004	-
6	0.109	0.090	0.056	0.053	0.035	-0.019	-
7	0.271	0.214	0.029	0.242	0.185	-0.057	-
8	0.241	0.156	0.093	0.148	0.063	-0.086	-
10	0.973	0.925	0.038	0.935	0.887	-0.048	-
11	0.073	0.070	0.023	0.050	0.048	-0.002	-
12	0.050	0.061	0.045	0.005	0.016	0.011	-
13	0.845	0.996	0.071	0.775	0.925	0.151	+
14	0.054	0.076	0.090	-0.036	-0.015	0.022	-
15	1.652	1.470	0.030	1.622	1.440	-0.183	-
16	0.116	0.087	0.026	0.090	0.061	-0.029	-
17	0.068	0.054	0.027	0.041	0.027	-0.014	-
18	0.060	0.065	0.042	0.018	0.023	0.005	-
19	0.088	0.029	0.035	0.053	-0.006	-0.059	-
20	0.112	0.079	0.073	0.039	0.005	-0.034	-
21	0.095	0.042	0.023	0.072	0.020	-0.053	-
22	0.627	0.529	0.026	0.601	0.503	-0.099	-
23	0.069	0.052	0.049	0.020	0.003	-0.017	-
24	0.080	0.082	0.049	0.031	0.033	0.002	-
26	0.053	0.036	0.028	0.025	0.008	-0.017	-
27	0.238	0.300	0.032	0.206	0.269	0.063	+
28	0.063	0.053	0.059	0.004	-0.006	-0.009	-
29	1.414	0.784	0.068	1.346	0.716	-0.630	-
30	1.301	1.112	0.068	1.233	1.044	-0.189	-
31	0.460	0.280	0.063	0.397	0.217	-0.180	-
32	0.091	0.071	0.040	0.052	0.032	-0.020	-
33	0.104	0.121	0.111	-0.007	0.010	0.017	-
35	0.179	0.217	0.084	0.095	0.133	0.039	-
36	0.077	0.108	0.086	-0.008	0.023	0.031	-
37	0.072	0.075	0.078	-0.006	-0.004	0.003	-
38	0.053	0.056	0.064	-0.011	-0.008	0.003	-
39	0.062	0.063	0.075	-0.013	-0.012	0.001	-
40	0.121	0.094	0.085	0.036	0.009	-0.027	-
41	0.077	0.051	0.036	0.041	0.016	-0.026	-
42	0.092	0.066	0.028	0.065	0.039	-0.026	-
43	0.098	0.069	0.038	0.060	0.031	-0.029	-
44	0.076	0.067	0.037	0.039	0.030	-0.009	-
45	0.076	0.089	0.090	-0.014	-0.002	0.013	-
46	0.124	0.084	0.045	0.079	0.039	-0.041	-
47	0.072	0.088	0.050	0.022	0.039	0.017	-
49	0.122	0.147	0.031	0.092	0.116	0.025	-
50	0.700	1.106	0.066	0.634	1.040	0.407	+
51	0.147	0.097	0.028	0.120	0.070	-0.050	-
52	0.050	0.042	0.032	0.018	0.010	-0.008	-
53	0.660	0.648	0.044	0.616	0.604	-0.012	-
54	0.702	0.729	0.051	0.652	0.678	0.027	-
55	0.386	0.344	0.064	0.322	0.280	-0.042	-
56	0.073	0.060	0.035	0.038	0.026	-0.013	-

Gamma interferon results - 24 months, Aug 2002

SheepID	Optical densities			a-c	j-c	j-a	Result*
	av	jo	c				
57	0.196	0.245	0.049	0.147	0.196	0.049	-
58	1.842	1.997	0.051	1.792	1.946	0.155	+
59	0.066	0.067	0.061	0.005	0.006	0.001	-
60	0.102	0.070	0.053	0.050	0.018	-0.032	-
61	0.131	0.200	0.024	0.107	0.176	0.069	+
62	0.083	0.063	0.027	0.057	0.037	-0.020	-
63	0.202	0.130	0.050	0.153	0.080	-0.073	-
64	0.060	0.079	0.058	0.002	0.022	0.020	-
65	0.902	0.865	0.028	0.874	0.838	-0.037	-
66	0.059	0.050	0.058	0.001	-0.008	-0.009	-
67	0.172	0.118	0.043	0.130	0.075	-0.055	-
68	0.061	0.081	0.049	0.013	0.032	0.020	-
69	0.109	0.112	0.033	0.076	0.079	0.003	-
70	0.268	0.187	0.026	0.242	0.162	-0.081	-
71	0.048	0.044	0.060	-0.012	-0.016	-0.004	-
72	0.107	0.120	0.048	0.059	0.072	0.013	-
73	0.038	0.032	0.048	-0.011	-0.017	-0.006	-
74	0.631	0.975	0.093	0.538	0.882	0.344	+
75	0.099	0.116	0.091	0.008	0.025	0.017	-
76	1.321	1.548	0.027	1.294	1.521	0.227	+
77	0.069	0.084	0.080	-0.012	0.004	0.016	-
78	1.041	1.353	0.058	0.983	1.295	0.312	+
79	0.084	0.083	0.049	0.035	0.034	-0.002	-
80	0.063	0.058	0.045	0.018	0.013	-0.005	-
81	0.121	0.123	0.067	0.055	0.056	0.002	-
90	0.071	0.051	0.019	0.052	0.032	-0.020	-

* Positive if OD(Johnin) - OD(Avian) > 0.05

Gamma interferon results - 30 months, Mar 2003

SheepID	Optical densities			a-c	j-c	j-a	Result*
	av	jo	c				
1	0.210	0.402	0.026	0.184	0.376	0.192	+
2	0.057	0.078	0.035	0.022	0.043	0.021	-
4	0.151	0.148	0.038	0.113	0.110	-0.003	-
5	0.066	0.106	0.032	0.034	0.074	0.040	-
6	0.102	0.124	0.035	0.067	0.089	0.022	-
7	0.405	0.409	0.071	0.334	0.338	0.004	-
8							
10	0.749	1.115	0.089	0.660	1.026	0.366	+
11	0.128	0.161	0.058	0.070	0.103	0.033	-
12	0.125	0.322	0.043	0.082	0.279	0.197	+
13	1.913	1.799	0.057	1.856	1.742	-0.114	-
14	0.051	0.071	0.053	-0.002	0.018	0.020	-
15	1.280	2.108	0.056	1.224	2.052	0.828	+
16	0.312	0.351	0.099	0.213	0.252	0.039	-
17	0.182	0.329	0.073	0.109	0.256	0.147	+
18	0.047	0.067	0.031	0.016	0.036	0.020	-
19	0.109	0.137	0.078	0.031	0.059	0.028	-
20	0.117	0.052	0.044	0.073	0.008	-0.065	-
21	0.312	0.191	0.033	0.279	0.158	-0.121	-
22							
23	1.934	3.854	0.067	1.867	3.787	1.920	+
24	0.150	0.134	0.177	-0.027	-0.043	-0.016	-
26	0.181	0.141	0.238	-0.057	-0.097	-0.040	-
27	0.269	0.687	0.031	0.238	0.656	0.418	+
28	0.060	0.073	0.059	0.001	0.014	0.013	-
29	0.053	0.047	0.024	0.029	0.023	-0.006	-
30	1.215	1.776	0.082	1.133	1.694	0.561	+
31	0.251	0.317	0.041	0.210	0.276	0.066	+
32	0.195	0.267	0.037	0.158	0.230	0.072	+
33	0.098	0.134	0.089	0.009	0.045	0.036	-
35	0.204	0.260	0.026	0.178	0.234	0.056	+
36	0.137	0.236	0.084	0.053	0.152	0.099	+
37	0.056	0.109	0.027	0.029	0.082	0.053	+
38	0.043	0.052	0.036	0.007	0.016	0.009	-
39	0.053	0.107	0.036	0.017	0.071	0.054	+
40	0.095	0.089	0.026	0.069	0.063	-0.006	-
41	0.086	0.143	0.030	0.056	0.113	0.057	+
42	0.131	0.186	0.067	0.064	0.119	0.055	+
43	0.262	0.144	0.078	0.184	0.066	-0.118	-
44	0.171	0.347	0.031	0.140	0.316	0.176	+
45	0.039	0.053	0.025	0.014	0.028	0.014	-
46	1.062	1.285	0.126	0.936	1.159	0.223	+
47	0.094	0.106	0.110	-0.016	-0.004	0.012	-
49	0.555	1.074	0.058	0.497	1.016	0.519	+
50	0.736	1.216	0.223	0.513	0.993	0.480	+
51	0.397	0.431	0.042	0.355	0.389	0.034	-
52	0.283	0.370	0.033	0.250	0.337	0.087	+
53							
54							
55							
56	0.285	0.540	0.031	0.254	0.509	0.255	+

Gamma interferon results - 30 months, Mar 2003

SheepID	Optical densities			a-c	j-c	j-a	Result*
	av	jo	c				
57	0.242	0.244	0.106	0.136	0.138	0.002	-
58	1.256	1.500	0.040	1.216	1.460	0.244	+
59	0.103	0.110	0.072	0.031	0.038	0.007	-
60	0.529	1.053	0.076	0.453	0.977	0.524	+
61	0.155	0.317	0.030	0.125	0.287	0.162	+
62	0.170	0.139	0.038	0.132	0.101	-0.031	-
63	0.209	0.322	0.029	0.180	0.293	0.113	+
64	0.162	0.232	0.053	0.109	0.179	0.070	+
65	0.462	0.454	0.041	0.421	0.413	-0.008	-
66	0.237	0.252	0.139	0.098	0.113	0.015	-
67	0.292	0.335	0.081	0.211	0.254	0.043	-
68							
69	0.355	0.508	0.041	0.314	0.467	0.153	+
70	0.332	0.354	0.055	0.277	0.299	0.022	-
71	0.095	0.152	0.029	0.066	0.123	0.057	+
72	1.117	1.229	0.131	0.986	1.098	0.112	+
73	0.029	0.049	0.026	0.003	0.023	0.020	-
74							
75	0.167	0.151	0.030	0.137	0.121	-0.016	-
76							
77	0.149	0.164	0.084	0.065	0.080	0.015	-
78	0.110	0.131	0.064	0.046	0.067	0.021	-
79	0.047	0.067	0.055	-0.008	0.012	0.020	-
80	0.239	0.388	0.053	0.186	0.335	0.149	+
81	0.253	0.298	0.035	0.218	0.263	0.045	-
90	0.095	0.154	0.029	0.066	0.125	0.059	+

* Positive if OD(Johnin) - OD(Avian) > 0.05

Gamma interferon results - 30 months, Mar 2003

SheepID	Optical densities			a-c	j-c	j-a	Result*
	av	jo	c				
1	0.083	0.087	0.043	0.040	0.044	0.004	-
2	0.088	0.086	0.119	-0.031	-0.033	-0.002	-
4	0.171	0.144	0.256	-0.085	-0.112	-0.027	-
5	0.113	0.119	0.099	0.014	0.020	0.006	-
6	0.082	0.08	0.11	-0.028	-0.030	-0.002	-
7	0.068	0.083	0.066	0.002	0.017	0.015	-
8							
10	0.098	0.208	0.054	0.044	0.154	0.110	+
11	0.135	0.163	0.153	-0.018	0.010	0.028	-
12	0.054	0.073	0.051	0.003	0.022	0.019	-
13							
14	0.067	0.077	0.064	0.003	0.013	0.010	-
15	0.098	0.097	0.081	0.017	0.016	-0.001	-
16	0.083	0.096	0.097	-0.014	-0.001	0.013	-
17	0.18	0.227	0.114	0.066	0.113	0.047	-
18	0.059	0.06	0.06	-0.001	0.000	0.001	-
19	0.106	0.102	0.078	0.028	0.024	-0.004	-
20	0.049	0.051	0.059	-0.010	-0.008	0.002	-
21	0.073	0.072	0.05	0.023	0.022	-0.001	-
22							
23	0.076	0.173	0.062	0.014	0.111	0.097	+
24	0.064	0.064	0.092	-0.028	-0.028	0.000	-
26	0.121	0.135	0.105	0.016	0.030	0.014	-
27	0.19	0.257	0.108	0.082	0.149	0.067	+
28	0.073	0.085	0.069	0.004	0.016	0.012	-
29							
30	0.184	0.258	0.069	0.115	0.189	0.074	+
31	0.128	0.288	0.05	0.078	0.238	0.160	+
32	0.12	0.195	0.105	0.015	0.090	0.075	+
33	0.076	0.102	0.077	-0.001	0.025	0.026	-
35	0.096	0.087	0.062	0.034	0.025	-0.009	-
36	0.123	0.163	0.071	0.052	0.092	0.040	-
37	0.081	0.118	0.064	0.017	0.054	0.037	-
38	0.049	0.048	0.043	0.006	0.005	-0.001	-
39	0.085	0.097	0.068	0.017	0.029	0.012	-
40	0.127	0.115	0.129	-0.002	-0.014	-0.012	-
41	0.096	0.105	0.09	0.006	0.015	0.009	-
42	0.087	0.108	0.053	0.034	0.055	0.021	-
43	0.116	0.15	0.082	0.034	0.068	0.034	-
44	0.066	0.065	0.072	-0.006	-0.007	-0.001	-
45	0.075	0.075	0.071	0.004	0.004	0.000	-
46	0.425	0.42	0.205	0.220	0.215	-0.005	-
47	0.064	0.064	0.055	0.009	0.009	0.000	-
49	0.107	0.296	0.072	0.035	0.224	0.189	+
50	0.182	0.296	0.088	0.094	0.208	0.114	+
51	0.072	0.093	0.078	-0.006	0.015	0.021	-
52	0.173	0.994	0.15	0.023	0.844	0.821	+
53							
54							
55							
56	0.059	0.087	0.041	0.018	0.046	0.028	-

Gamma interferon results - 30 months, Mar 2003

SheepID	Optical densities			a-c	j-c	j-a	Result*
	av	jo	c				
57	0.137	0.132	0.128	0.009	0.004	-0.005	-
58	0.228	0.291	0.077	0.151	0.214	0.063	+
59	0.083	0.074	0.074	0.009	0.000	-0.009	-
60	0.057	0.064	0.068	-0.011	-0.004	0.007	-
61	0.089	0.11	0.075	0.014	0.035	0.021	-
62	0.101	0.13	0.105	-0.004	0.025	0.029	-
63	0.053	0.06	0.052	0.001	0.008	0.007	-
64	0.088	0.081	0.092	-0.004	-0.011	-0.007	-
65	0.175	0.179	0.048	0.127	0.131	0.004	-
66	0.064	0.066	0.076	-0.012	-0.010	0.002	-
67	0.085	0.095	0.069	0.016	0.026	0.010	-
68							
69	0.103	0.13	0.109	-0.006	0.021	0.027	-
70	0.104	0.838	0.06	0.044	0.778	0.734	+
71	0.083	0.09	0.074	0.009	0.016	0.007	-
72	0.099	0.098	0.065	0.034	0.033	-0.001	-
73	0.079	0.071	0.122	-0.043	-0.051	-0.008	-
74							
75	0.052	0.062	0.044	0.008	0.018	0.010	-
76							
77	0.12	0.146	0.129	-0.009	0.017	0.026	-
78							
79	0.048	0.062	0.052	-0.004	0.010	0.014	-
80	0.081	0.111	0.054	0.027	0.057	0.030	-
81	0.115	0.139	0.112	0.003	0.027	0.024	-
90	0.061	0.065	0.08	-0.019	-0.015	0.004	-

* Positive if OD(Johnin) - OD(Avian) > 0.05

Appendix 2f. Results for faecal examinations: culture* and direct PCR

Sheep ID	9 mth		12 mth		18 mth		24 mth		30 mth		36 mth		OJD status	Age at death		
	IFC	dpcr	IFC	dpcr	IFC	dpcr	IFC	dpcr	IFC	dpcr	IFC	dpcr				
1	-	-	-	-	-	-	-	inc	c	-	-	-	recovered			
2	-	-	-	+	-	-	-	-	-	-	-	-	mild OJD			
4	-	+	-	-	-	-	-	-	-	-	-	-	recovered			
5	-	-	-	-	-	-	-	-	-	-	+	33	+	mild OJD		
6	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD			
7	+	41	-	inc	-	-	-	-	-	-	c	-	recovered			
8	-	-	-	-	+	24	+	+	20	+	-	-	died OJD	24m		
10	-	-	-	-	-	-	-	-	+	40	+	+	28	+	died OJD	35m
11	-	-	-	-	-	-	-	-	-	-	-	inc	uninfected/n			
12	-	-	-	-	-	-	-	-	-	-	-	inc	uninfected/r			
13	-	-	-	-	+	50	-	+	32	+	+	32	+	died OJD	29m	
14	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD			
15	-	-	-	-	-	-	-	+	+	48	-	+	34	+	severe OJD	
16	-	-	-	inc	-	-	-	-	-	-	-	-	mild OJD			
17	-	-	-	-	-	-	-	-	-	-	+	38	+	severe OJD		
18	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n			
19	-	-	-	-	-	-	-	-	-	-	-	inc	mild OJD			
20	-	-	-	-	-	-	-	-	c	-	-	-	uninfected/n			
21	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n			
22	-	-	-	-	+	28	+	+	25	+	-	-	dead, mild OJ	24m		
23	-	-	-	-	-	-	-	-	+	35	+	+	25	+	mild OJD	
24	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n			
26	-	-	-	-	-	-	-	-	-	-	+	31	+	mild OJD		
27	-	-	-	-	-	-	-	-	-	-	+	35	+	mild OJD		
28	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n			
29	-	-	-	-	+	39	+	+	26	+	+	18	+	died OJD	29m	
30	-	-	+	41	inc	-	-	-	-	c	-	-	inc	recovered		
31	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD			
32	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r			
33	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n			
35	-	-	-	inc	-	-	-	-	-	-	-	-	mild OJD			
36	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD			
37	-	-	-	inc	-	-	-	-	-	-	-	-	mild OJD			
38	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n			
39	-	-	-	-	-	-	-	c	-	-	-	-	mild OJD			
40	-	-	-	+	-	-	-	-	-	-	-	-	recovered			
41	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD			

Appendix 2f. Results for faecal examinations: culture* and direct PCR

Sheep ID	9 mth		12 mth		18 mth		24 mth		30 mth		36 mth		OJD status	Age at death
	IFC	dpcr	IFC	dpcr	IFC	dpcr	IFC	dpcr	IFC	dpcr	IFC	dpcr		
42	-	-	-	inc	-	-	-	-	-	-	-	-	mild OJD	
43	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
44	-	-	-	inc	-	-	-	-	-	inc	-	-	mild OJD	
45	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
46	-	-	-	-	-	-	+ 39	-	+ 31	+	+ 18	+	died OJD	33m
47	-	-	-	-	-	-	-	-	c	-	-	-	mild OJD	
49	-	-	-	-	-	-	-	-	-	-	+ 31	+	severe OJD	
50	+ 35	-	-	-	-	-	-	-	-	-	-	-	severe OJD	
51	+ 50	-	-	-	-	-	c	-	-	-	-	-	recovered	
52	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
53	-	-	-	-	-	-	+ 38	+	-	-	-	-	dead, severe	25m
54	+ 50	inc	-	-	-	inc	+ 40	inc	-	-	-	-	died OJD	28m
55	-	-	-	-	+ 39	-	+ 28	+	-	-	-	-	died OJD	24m
56	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
57	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
58	-	-	-	-	-	-	+ 34	+	+ 36	inc	+ 29	+	died OJD	34m
59	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
60	-	-	-	-	-	-	-	-	+ 37	inc	+ 22	+	severe OJD	
61	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
62	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
63	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
64	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
65	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
66	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
67	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
68	-	-	-	-	-	-	-	-	-	-	-	-	dead, uninfected	25m
69	-	-	-	-	-	-	-	-	-	inc	-	-	uninfected/r	
70	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
71	-	-	-	-	-	-	-	-	-	-	+ 31	+	severe OJD	
72	+ 29	+	-	-	-	-	-	-	-	-	-	inc	recovered	
73	+ 50	-	-	-	-	-	-	-	-	-	-	-	recovered	
74	-	-	-	-	-	-	+ 31	+	-	-	-	-	died OJD	28m
75	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
76	+ 27	+	-	-	+ 39	-	-	-	-	-	-	-	died OJD	24m
77	+ 50	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
78	-	-	-	-	+ 38	-	+ 39	+	+ 25	+	-	-	died OJD	29m
79	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	

Appendix 2f. Results for faecal examinations: culture* and direct PCR

Sheep ID	9 mth		12 mth		18 mth		24 mth		30 mth		36 mth		OJD status	Age at death
	IFC	dpcr	IFC	dpcr	IFC	dpcr	IFC	dpcr	IFC	dpcr	IFC	dpcr		
80	-	-	-	+	-	-	-	-	-	-	-	inc	mild OJD	
81	-	-	-	-	-	-	-	-	-	-	-	inc	uninfected/r	
90	-	-	-	-	-	-	-	-	c	-	-	-	uninfected/r	
Total +ve	8	3	1	3	7	3	9	8	6	3	9	9		
Total sheep	77	77	77	77	77	77	74	74	66	66	63	63		
% +ve	10	3.9	1.3	3.9	9.1	3.9	12	11	9.1	4.5	14	14		
For analysis:	8/74	3/74	1/74	3/74	6/74	2/74	7/71	6/71	6/66	3/66	9/63	9/63		

* Days taken for the cumulative growth index in bactec culture to reach 1000 are indicated for confirmed positive cultures

These sheep excluded from statistical analysis

Samples collected at necropsy. Not included in % positive, total sheep, nor in statistical analysis

Appendix 2g. Histopathology, details, Sep 2002 (12 months)

Sheep ID	Mn/no	Gut H&E- ZN-gt	Node H&E-i ZN-nr	Combined H&E- ZN-st	Perez	Gut Comment-gut	Node Comment-node		
1	01/6608	-	-	-	-				
2	01/6190	-	-	-	-				
4	01/7788	-	-	-	-				
5	01/6858	-	-	-	-				
6	01/7788	?	-	?	-	?	equivocal macrophage focus in lp equivocal macrophage focus pcortex		
7	01/7400	-	?	-	?	-	?	equivocal macrophage infiltrates in sc sinus, pigment-laden macrophage clumps, equivocal foal necrosis (oat cells), 2 equivocal granulomas (probably remnants/hyaline degeneration of germinal centres)	
8	01/7400	-	?	-	?	-	?		
10	01/7400	-	-	-	-	-	-		
11	01/7624	-	-	?	-	?	-	?	equivocal macrophage focus pcortex
12	01/7400	?	-	?	-	?	-	?	singe equivocal macrophage clump in villous tip equivocal granuloma in pcortex, some oedema/necrosis?? With oat cells
13	01/6858	-	-	-	-	-	-	-	poor section poor section
14	01/7624	-	-	-	-	-	-	-	
15	01/7990	-	-	?	-	?	-	?	equivocal macrophage focus pcortex
16	01/7400	-	-	-	-	-	-	-	
17	01/7624	-	-	-	-	-	-	-	poor gut sections single culump of pigment-laden macrophages
18	01/6608	-	-	-	-	-	-	-	
19	01/6190	?	-	?	-	?	-	?	very equivocal lesions in dome areas equivocal macrophage focus pcortex
20	01/7990	-	-	-	-	-	-	-	
21	01/7400	-	-	-	-	-	-	-	
22	01/7788	-	-	-	-	-	-	-	
23	01/6190	-	-	-	-	-	-	-	
24	01/7624	-	-	-	-	-	-	-	poorly oriented gut sections
26	01/7990	-	-	-	-	-	-	-	barely legible block
27	01/6983	-	-	?	-	?	-	?	equivocal macrophage focus pcortex
28	01/6983	?	-	?	-	?	-	?	equivocal macrophage clump in interfollicular PP equivocal macrophage focus pcortex
29	01/7624	-	-	-	-	-	-	-	note: confusion between 2 X 29 sections (either one could be 69)
30	01/7788	-	-	+	++	+	++	2n	Severasl highly suspiciuos macrophage foci pcortex
31	01/7400	-	-	?	-	?	-	?	equivocal very ill-defined chnages (? necrosis or ill-defined macrophage clumps)
32	01/7788	-	-	-	-	-	-	-	
33	01/6983	-	-	-	-	-	-	-	
35	01/6858	-	-	-	-	-	-	-	
36	01/7990	-	-	-	-	-	-	-	
37	01/6983	-	-	-	-	-	-	-	clumps of pigment-laden macrophages in pcortex
38	01/7400	-	-	-	-	-	-	-	
39	01/7624	-	-	?	-	?	-	?	poorly oriented gut sections equivocal macrophage focus pcortex (tending to pigmented)
40	01/6608	-	-	-	-	-	-	-	
41	01/7990	-	-	-	-	-	-	-	
42	01/6983	?	-	-	-	?	-	?	singe equivocal macrophage clump in villous tip
43	01/7990	-	-	?	-	?	-	?	equivocal macrophage focus pcortex
44	01/7990	-	-	-	-	-	-	-	note: confusion between 44 and 71
45	01/7990	-	-	-	-	-	-	-	
46	01/6608	-	-	-	-	-	-	-	
47	01/6983	-	-	-	-	-	-	-	
49	01/6608	-	-	-	-	-	-	-	

Appendix 2g. Histopathology, details, Sep 2002 (12 months)

Sheep ID	Mn/no	Gut		Node		Combined		Perez	Gut Comment-gut	Node Comment-node
		H&E-	ZN-gt	H&E-i	ZN-n	H&E-	ZN-st			
50	01/7990	-	-	-	-	-	-	-	barely legible	
51	01/6858	-	-	-	-	-	-	-	poor section	
52	01/6868	-	-	-	-	-	-	-		
53	01/6608	?	-	-	-	?	-	?	equivocal macrophage clumps in interfollicular PP	
54	01/7990	+	+	+	++	+	++	2	small macrophage foci in lp & if, barely legible block	Severasl highly suspiciuos macrophage foci pcortex
55	01/6608	-	-	-	-	-	-	-		
56	01/6868	-	-	-	-	-	-	-	note: confusion between 56 and 66	
57	01/6190	-	-	-	-	-	-	-	little PP in section	
58	01/7400	-	-	-	-	-	-	-	some crypt loss, villous fusion (post coccidia?)	
59	01/7788	-	-	-	-	-	-	-		
60	01/6983	?	-	-	-	?	-	?	suspicious focus in a PP follicle	
61	01/7400	-	-	-	-	-	-	-		
62	01/7624	-	-	-	-	-	-	-		single clump of pigment-laden macrophages
63	01/7624	+	-	+	-	+	-	1	highly suspicious macrophage clump in follicular remnant	Severasl highly suspiciuos macrophage foci pcortex & subcapsular area
64	01/7788	-	-	-	-	-	-	-	coccidial granuloma in PP	
65	01/7624	-	-	-	-	-	-	-	PP has morphology of ICV or jejunal PP	
66	01/6868	-	-	-	-	-	-	-	note: confusion between 56 and 66	
67	01/6983	-	-	-	-	-	-	-		
68	01/7788	-	-	-	-	-	-	-	poor gut sections	
69	01/7624	-	-	-	-	-	-	-	note: confusion between 2 X 29 sections (either one could be 69)	foci of possible extramedullary haematopoeisis
70	01/7400	-	-	-	-	-	-	-		
71	01/7990	-	-	-	-	-	-	-	note: confusion between 44 and 71	
72	01/6190	-	-	-	-	-	-	-		
73	01/7990	-	-	-	-	-	-	-		
74	01/6983	-	-	?	-	?	-	?		doubtful focus in a follicular remnant (some necrosis also)
75	01/7788	-	-	-	-	-	-	-		
76	01/7788	+	+	+	+	+	+	2	typical multifocal lesions in PP and lp	multifocal granulomas pcortex, ext to medullary cords, some giant cells
77	01/7788	-	-	-	-	-	-	-		
78	01/6190	-	-	-	-	-	-	-		
79	01/6858	-	-	-	-	-	-	-		
80	01/6190	-	-	-	-	-	-	-		
81	01/6858	-	-	-	-	-	-	-		
90	01/6858	-	-	-	-	-	-	-	poor section	poor section

Appendix 2g. Histopathology, details, biopsies Feb 2002 (18 months)

Sheep ID	Mn/no	Gut		Node		Sum		Perez	Gut Comment	Node Comment	
		H&E	ZN	H&E	ZN	H&E	ZN				
1	02-2395	-	-	-	-	-	-	nsf		nsf	
2											ns
4											ns
5											ns
6											ns
7	02-2395	-		?	-	?	-	?	nsf	scattered foci of ill-defined macrophages.	
8	02-2179	+	+++	+	-	+	+++	3b	severe multifocal	scattered foci	
10											ns
11											ns
12											ns
13											ns
14											ns
15											ns
16											ns
17											ns
18											ns
19											ns
20											ns
21											ns
22											ns
23	02-2395	-	-	-	-	-	-	-	nsf		nsf
24											ns
26											ns
27	02-2395	+	+	+	-	+	+	3c	multifocal macrophage clumps, diffuse lymphoid infiltration,villous fusion, crypt abscesses	severe multifocal	
28											ns
29											ns
30	02-2395	+	-	-	-	+	-	3a	scattered macrophage clumps, severe diffuse lymphoid infiltrate, no villous blunting	nsf	
31	02-2179	?	-	-	-	?	-	?	some equivocal macrophage foci, cocci	nsf	
32											ns
33											ns
35	02-2395	?	-	-	-	?	-	?	some equivocal macrophage foci, cocci	nsf	
36											ns
37											ns
38											ns
39											ns
40											ns
41											ns
42											ns
43											ns
44											ns
45											ns
46											ns
47											ns
49											ns

Appendix 2g. Histopathology, details, biopsies Feb 2002 (18 months)

Sheep ID	Mn/no	Gut		Node		Sum		Perez	Gut Comment	Node Comment
		H&E	ZN	H&E	ZN	H&E	ZN			
50	02-2395	+	-	+	-	+	-	3c	multifocal macrophage clumps, diffuse lymphoid infiltration, villous fusion, crypt abscesses	multifocal ep. macrophage clumps
51	02-2395	+	-	-	-	+	-	1	multifocal suspicious macrophage clumps in PP	nsf
52										ns
53										ns
54	02-1992	+	+	+	?	+	+	3c	severe multifocal to diffuse, few afb, lymphangitis	severe multifocal with occ. Giant cells
55										ns
56										ns
57										ns
58	02-2395	-		-		-			nsf	nsf
59										ns
60										ns
61										ns
62										ns
63	02-2395	-		-		-			nsf	nsf
64										ns
65	02-1992	-		-		-			nsf	nsf
66										ns
67										ns
68										ns
69										ns
70	02-2179	?	-	?	-	?	-	?	equivocal macrophage focus	equivocal macrophage focus
71										ns
72	02-2395	-		?		?		?	nsf	granulomas with mineralised contents
73	02-2395	-		-		-			nsf	nsf
74										ns
75										ns
76	02-2395	+	+	+	-	+	+	3c	multifocal macrophage clumps, diffuse lymphoid infiltration, villous fusion, few afb	severe multifocal
77	02-2395	-		-		-			nsf	nsf
78	02-2395	?	-	+	+	+	+	2n	focal granulomas in submucosa, some mineralised	multifocal ep. macrophage clumps, giant cells
79										ns
80										ns
81										ns
90										ns

ns = not sampled

Appendix 2g. Histopathology, details, biopsies Aug 2002 (24 months)

Sheep ID	Mn/no	Gut		Node		Sum		perez	Gut Comment	Node Comment
		H&E	ZN	H&E	ZN	H&E	ZN			
1	02/7954	-	-	-	-	-	-	-	nsf	nsf
2	02/8361	+	+	-	-	+	+	2	multifocal PP lesions, equivocal in mucosa	nsf
4	02/8203	-	-	-	-	-	-	-	nsf, widespread inflam, pigmented macrophage clumps	nsf, pigmented macrophage clumps
5	02/8361	-	-	-	-	-	-	-	nsf	nsf
6	02/7954	-	-	-	-	-	-	-	nsf	nsf
7	02/8203	+	-	+	-	+	-	1	focal lesions in interfol PP	single suspicious focus
8	02-6916	+	+++	+	+	+	+++	3b	severe diffuse multibacillary	severe multifocal to diffuse, occ AFB
10	02/7954	+	+	+	-	+	+	3c	multifocal to diffuse, villous fusion, mixed inflam, few single AFB	multifocal
11	02/7954	-	-	-	-	-	-	-	nsf	nsf
12	02/7954	-	-	-	-	-	-	-	nsf	nsf
13	02/7954	+	+++	+	+	+	+++	3b	severe diffuse multibacillary	severe multifocal to diffuse, occ AFB
14	02/8203	-	-	-	-	-	-	-	nsf	nsf
15	02/8361	+	+	+	-	+	+	3c	severe multifocal, villous fusion, mixed inflam, few ZN positive clumps	multifocal
16	02/8416	-	-	-	-	-	-	-	nsf	nsf
17	02/8203	-	-	-	-	-	-	-	nsf	nsf
18	02/7954	-	-	-	-	-	-	-	nsf	nsf
19	02/7954	-	-	-	-	-	-	-	nsf	nsf
20	02/8361	-	-	-	-	-	-	-	nsf	nsf
21	02/8203	-	-	-	-	-	-	-	nsf	nsf, pigmented macrophage clumps
22	ns									
23	02/8416	-	-	-	-	-	-	-	nsf	nsf
24	02/8203	-	-	-	-	-	-	-	nsf	nsf
26	02/8203	-	-	-	-	-	-	-	nsf	nsf
27	02/7954	+	-	+	-	+	-	3a	multifocal in PP and mucosa, mild mixed inflam infiltrate, equivocal villous blunting	nsf
28	02/7954	-	-	-	-	-	-	-	nsf	nsf, haemato/granulopoesis in medullary sinuses
29	02/8361	+	+++	+	-	+	+++	3b	severe diffuse multibacillary, severe lymphangitis	severe multifocal to diffuse
30	02/7954	-	-	-	-	-	-	-	nsf	nsf
31	02/7954	-	-	-	-	-	-	-	nsf, granulomas in PP with refractile granules	nsf
32	02/8203	-	-	-	-	-	-	-	nsf	nsf
33	02/8361	-	-	-	-	-	-	-	nsf	nsf
35	02/8416	+	-	-	-	+	-	1	widespread multifocal lesions in PP	nsf, some coccidia.
36	02/7954	-	-	-	-	-	-	-	nsf	nsf
37	02/8203	-	-	-	-	-	-	-	nsf	nsf, pigmented macrophage clumps
38	02/8203	-	-	-	-	-	-	-	nsf	nsf
39	02/8203	-	-	-	-	-	-	-	nsf	nsf

Appendix 2g. Histopathology, details, biopsies Aug 2002 (24 months)

Sheep ID	Mn/no	Gut		Node		Sum		perez	Gut Comment	Node Comment
		H&E	ZN	H&E	ZN	H&E	ZN			
40	02/8416	?	-	-	-	?	-	?	single granuloma with giant cells in PP, a bit equivocal	nsf
41	02/7954	-	-	-	-	-	-	-	nsf	nsf
42	02/8361	-	-	-	-	-	-	-	nsf	nsf
43	02/8361	-	-	-	-	-	-	-	nsf	nsf, focal caseous/hyaline granuloma with giant cells (likely parasitic)
44	02/8203	-	-	-	-	-	-	-	nsf, pigmented macrophage clump in PP	nsf
45	02/8203	-	-	-	-	-	-	-	nsf, granulocytic infiltr in PP, crypt loss (post cocci?)	nsf
46	02/8203	+	+	+	-	+	+	3a	multifocal in PP and mucosa, mild mixed inflam infiltrate	focal lesions
47	02/8203	-	-	-	-	-	-	-	nsf	nsf, pigmented macrophage clumps
49	02/7954	-	-	-	-	-	-	-	nsf, granulomas in PP with refractile granules	nsf
50	02/8203	+	-	+	-	+	-	3c	diffuse lymphoid infiltration, villous blunting, multifocal macrophage clumps, typical paucibacillary	mild multifocal
51	02/8203	-	-	-	-	-	-	-	nsf	nsf
52	02/8203	+	-	-	-	+	-	1	focal lesions in interfol PP, mixed inflam in lp	nsf, subcapsular fibrosis ans eosinophil infiltr.
53	02/8361	+	+++	+	-	+	+++	3b	moderate diffuse multibacillary, AFB very pale	multifocal to diffuse
54	02/8416	+	-	+	-	+	-	3c	diffuse lymphoid infiltration, villous blunting, multifocal macrophage clumps (widespread but rather inconspicuous), classical paucibacillary	multifocal
55	02/8203	+	+++	+	+	+	+++	3b	severe diffuse multibacillary	multifocal, few AFB, faint
56	02/7954	-	-	-	-	-	-	-	nsf	nsf
57	02/7954	+	-	-	-	+	-	1	focal lesions in interfol PP, some pigmented	nsf
58	02/8203	+	-	+	-	+	-	3c	diffuse lymphoid infiltration, villous blunting, multifocal macrophage clumps, typical paucibacillary	mild multifocal
59	02/8203	-	-	-	-	-	-	-	nsf	nsf
60	02/8203	-	-	-	-	-	-	-	nsf	nsf
61	02/8361	-	-	-	-	-	-	-	nsf	nsf
62	02/8361	-	-	-	-	-	-	-	nsf	nsf
63	02/7954	-	-	-	-	-	-	-	nsf, granulomas in PP with refractile pigmented granules, cocci megaloschizonts	nsf
64	02/8416	-	-	-	-	-	-	-	nsf	nsf
65	02/7954	?	-	-	-	?	-	?	focal lesions in interfol PP, some pigmented, a bit equivocal	nsf
66	02/8361	-	-	-	-	-	-	-	nsf	nsf
67	02/7954	-	-	-	-	-	-	-	nsf	nsf, medullary fibrosis, caseated granulomas in capsule
68	02/8203	-	-	-	-	-	-	-	nsf	nsf
69	02/7954	-	-	-	-	-	-	-	nsf	nsf
70	02/8361	-	-	-	-	-	-	-	nsf	nsf
71	02/7954	-	-	-	-	-	-	-	nsf	nsf

Appendix 2g. Histopathology, details, biopsies Aug 2002 (24 months)

Sheep ID	Mn/no	Gut		Node		Sum		perez	Gut Comment	Node Comment
		H&E	ZN	H&E	ZN	H&E	ZN			
72	02/8203	-	-	-	-	-	-	-	nsf	nsf, focal pyogranuloma, oat cells
73	02/8416	-	-	-	-	-	-	-	nsf	nsf
74	02/8361	+	+++	+	-	+	+++	3b	severe diffuse multibacillary, AFB very pale	multifocal
75	02/8361	-	-	-	-	-	-	-	nsf	nsf
76	02-6916	+	-	+	-	+	-	3c	diffuse lymphoid infiltration, with villous blunting, multifocal macrophage clumps, all AFB -ve, typical severe paucibacillary	moderate multifocal
77	02/8361	-	-	-	-	-	-	-	nsf	nsf
78	02/8416	+	++	+	-	+	++	3b/3c	mild diffuse mixed infiltration, villous blunting, multifocal macrophage clumps, looks like paucibacillary, but mod clumped AFB	mild multifocal
79	02/8203	-	-	-	-	-	-	-	nsf	nsf
80	02/8361	-	-	-	-	-	-	-	nsf	nsf
81	02/7954	-	-	-	-	-	-	-	nsf	nsf
90	02/8361	-	-	-	-	-	-	-	nsf	nsf, caseous/hyaline foci in heavy fibrous capsule

Results from these sheep are necropsy results. No Mock biopsy samples collected
 ns = not sampled (sheep died in paddock)

Appendix 2g. Histopathology and gross pathology, sheep euthanased Sep 03 (36 months)

Necropsy examination										Mock biopsy examination					Gross lesions							
ID	Mn/no-4	gut-h&e	gut-zn	node-h&e	node-zn	sum-zn	hist-36	gut-comment	node-comment	gut-h&e	gut-zn	node-h&e	node-zn	sum-zn	biops y summ	gross	ti	mln	lymph angiti s	surgery	site	fat reserv e
1	03/6791	-	-	-	-	-	-	suspicious lesions type 1 distribution, but with granualr refractile pigment obvious on ZN (biopsy segment only) , coccidia		?	-	-	-	-	?	-	-	-	-	-	minor adhes	good
2	03/6961	+	-	-	-	-	1			+	-	-	-	-	1	+	?	?	-	-	minor adhes	mod
4	03/6961	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-	mod/severe	good
5	03/6982	+	+	+	-	+	2			+	+	+	-	+	2	+	+	?	-	-	-	mod
6	03/6982	?	-	-	-	-	?	typical distribution in PP but all with refractile pigment		?	-	-	-	-	?	+	?	?	-	-	-	mod
7	03/6982	?	-	?	-	-	?	typical distribution in PP but all with refractile pigment		?	-	?	-	-	?	?	?	-	-	-	minor adhes	good
8	died OJD																					
10	died OJD																					
11	03/6961	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-	minor adhes	mod
12	03/6982	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-	mod adhesic	mod
13	died OJD																					
14	03/6961	+	-	+	-	-	1			+	-	+	-	-	1	-	-	-	-	-	minor adhes	mod
15	03/6791	+	+	+	-	+	3c	severe pauci,	multifocal, fibrosis, giant cells	+	+	+	-	+	3c	+	+++	+	+	-	-	mod
16	03/6791	+	-	+	-	-	1	ocassional PP foci, but most have pigment	typical multifocal, but also pigmented, some coccidia-assoc	+	-	+	-	-	1	?	?	-	-	-	-	good
17	03/6982	+	+	+	+	+	3c	typical, villous fusion mild in biopsy	typical multifocal 3/3 nodes	+	+	+	+	+	3c	+	?	+	-	-	-	mod
18	03/6982	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-	-	mod
19	03/6961	+	-	-	-	-	1			+	-	-	-	-	1	-	-	-	-	-	minor adhes	good
20	03/6982	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-	-	mod
21	03/6982	-	-	-	-	-	-			-	-	-	-	-	-	?	-	?	-	-	-	mod
22	died in paddock, no samples																					
23	03/6961	+	++	+	+	++	3a			+	+++	+	+	+++	3a	+	+++	si, ca:	+++	minor adhes	small	
24	03/6791	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-	-	good
26	03/6982	+	+	+	+	+	2			+	+	-	+	2	-	-	-	-	-	-	minor adhes	mod
27	03/6982	+	+	+	+	+	2			+	+	+	+	+	3c	+	-	+	-	-	minor adhes	mod
28	03/6961	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-	minor scarrir	mod
29	died OJD																					
30	03/6791	-	-	-	-	-	-		suspicious lesions, but may be coccidia-assoc (biopy section only)	-	-	?	-	-	?	+	?	-	?	-	minor adhes	good
31	03/6982	+	-	+	-	-	1			+	-	+	-	-	1	?	-	?	-	-	minor adhes	mod
32	03/6961	-	-	-	-	-	-			-	-	-	-	-	-	?	-	?	-	-	minor adhes	mod
33	03/6982	-	-	-	-	-	-			-	-	-	-	-	-	?	-	?	-	-	-	mod
35	03/6982	+	-	-	-	-	1			-	-	-	-	-	-	-	-	-	-	-	minor adhes	mod

Appendix 2g. Histopathology and gross pathology, sheep euthanased Sep 03 (36 months)
Necropsy examination

ID	Mn/no-4	Necropsy examination					hist-36	gut-commen	node-comment	Mock biopsy examination					Gross lesions							
		gut-h&e	gut-zn	node-h&e	node-zn	sum-zn				gut-h&e	gut-zn	node-h&e	node-zn	sum-zn	biops y summ	gross	ti	mln	lymph angiti s	surgery site	fat reserv e	
36	03/6961	-	-	+	-	-	2(n)			-	-	-	-	-	-	?	-	?	-	mod	adhesic	mod
37	03/6982	-		+	-	-	2(n)			+	-	+	-	-	2	?	-	?	-	-		mod
38	03/6982	-		-			-			-		-			-	-	-	-	-	minor	adhes	small
39	03/6791	?	-	?	-	-	?	suspicious PP foci, but generally with lots of pigment	suspicious foci, but generally with lots of pigment, some biopsy lesions have little or no pigment	-		+	-	-	2(n)	?	?	-	-	bodywall-run	good	
40	03/6982	?	-	?	-	-	?	pigmented clumps, eosinophilic enteritis jejunum	pigmented clumps	?	-	?	-	-	?	+	er jeju	?	-	-		mod
41	03/6961	-		-			-			-		-			-	-	-	-	-	minor	adhes	mod
42	03/6961	-		-			-			-		-			+	id ile	?	-	-	mod	adhesic	mod
43	03/6982	?	-	+	-	-	2(n)	pigmented clumps	typical + pigmented clumps (biopsy has only pigmented lesions0	?	-	?	-	-	?	?	-	?	-	minor	adhes	small
44	03/6982	-	-	?	-	-	?		clumps mostly pigmented, in biopsy only 2 highly pigmented clumps,	-	-	-	-	-	-	-	-	-	-	minor	adhes	mod
45	03/6982	-	-	-	-	-	-	single equivocal macrophage focus in lamina propria biopsy only. PP clear	highly pigmented clumps	-	-	-	-	-	-	?	?	-	-	minor	adhes	mod
46	died OJD																					
47	03/6961	?	-	-	-	-	?	type1 lesions in biopsy (some with pigment), in PM only in ICV and all heavily pigmented		+	-	-	-	-	1	-	-	-	-	minor	adhes	mod
49	03/6961	+	++	+	+	++	3b/3c			+	++	+	+	++	3b	+	+	++	+	minor	adhes	mod
50	03/6982	+	-	+	-	-	3c			+	-	+	-	-	3c	+	++	+	-	minor	adhes	mod
51	03/6982	-	-	-	-	-	-	heavily pigmented clumps	heavily pigmented clumps	-	-	-	-	-	-	+	?	?	-	minor	adhes	mod
52	03/6961	-		-			-		some heavily pigmented foci in PM only	-		-			-	-	-	-	-			mod
53	died in paddock, no samples																					
54	died OJD																					
55	died OJD																					
56	03/6961	-		+	-	-	2(n)		foci in node, some pigment, more and some typical in PM	-		?	-	-	?	?	-	?	-	minor	adhes	mod
57	03/6982	?	-	?	-	-	?	pigmented clumps	one equivocal focus	?	-	?	-	-	?	?	-	?	-			mod
58	died OJD																					
59	03/6961	-		-			-			-		-			-	-	-	-	-			mod

Appendix 2g. Histopathology and gross pathology, sheep euthanased Sep 03 (36 months)
Necropsy examination

ID	Mn/no-4	Necropsy examination					hist-36	gut-commen	node-comment	Mock biopsy examination					Gross lesions							
		gut-h&e	gut-zn	node-h&e	node-zn	sum-zn				gut-h&e	gut-zn	node-h&e	node-zn	sum-zn	biops y summ	gross	ti	mln	lymph angiti s	surgery site	fat reserv e	
60	03/6982	+	+++	+	+	+++	3b/3c	some areas 3c some 3b	severe multifocal + fibrosis	+	++	-	++	3a	+	+	?	-	minor adhes	mod		
61	03/6982	+	-	+	-	-	1	foci typical H&E, most have refractile granular pigment on ZN		+	-	+	-	-	1	-	-	-	-	minor adhes	small	
62	03/6982	-	-	-	-	-	-	densely pigmented foci in PP not seen in biopsy	densely pigmented foci	-	-	-	-	-	-	-	-	-	-	mod		
63	03/6961	?	-	?	-	-	?	pigmented clumps	pigmented clumps	?	-	?	-	-	?	?	-	?	-	minor adhes	mod	
64	03/6961	-	-	-	-	-	-			-	-	-	-	-	-	?	-	?	-	minor adhes	mod	
65	03/6982	?	-	-	-	-	?	pigmented clumps		?	-	-	-	-	?	-	-	-	-	minor adhes	mod	
66	03/6961	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	minor adhes	mod	
67	03/6982	?	-	-	-	-	?	pigmented clumps	heavily pigmented clumps	?	-	-	-	-	?	-	-	-	-	minor adhes	mod	
68	died in paddock, no samples																					
69	03/6961	-	-	-	-	-	-			-	-	-	-	-	-	?	-	?	-	mod adhesic	mod	
70	03/6982	-	-	-	-	-	-	highly pigmented clumps	pigmented clumps bioipsy only	-	-	?	-	-	?	-	-	-	-	mod		
71	03/6961	+	++	+	+	++	3b/3c	heavily pigmneted PP foci	pigmented foci only (biopsy), mineralised subcapsular foci with giant cells	+	+	+	+	+	3a/3c	+	-	+	-	minor adhes	mod	
72	03/6961	-	-	?	-	-	?			-	-	?	-	-	?	?	-	?	-	minor adhes	mod	
73	03/6961	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	mod		
74	died OJD																					
75	03/6961	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	mod		
76	died OJD															+	+	+	+			
77	03/6982	-	-	-	-	-	-	heavily pigmneted PP foci, one walled of debris-centred granuloma		-	-	-	-	-	-	?	-	?	-	minor adhes	mod	
78	died OJD																					
79	03/6791	-	-	-	-	-	-	a few foci, but pigment-laden	typical very few afb biosy only	-	-	+	+	+	2(n)	-	-	-	-	good		
80	03/6982	+	-	?	-	+	2			-	-	+	+	+	2(n)	+	?	?	+	mod adhesic	mod	
81	03/6961	-	-	?	-	-	?			-	-	-	-	-	-	+	?	+	-	mod adhesic	mod	
90	03/6791	-	-	-	-	-	-	single heavily pigmented focus		-	-	-	-	-	-	?	?	-0	-	good		

Appendix 2g. Gross and Histopathology, sheep dead before final sampling

ID	Mn/no-4	Necropsy details						node-comment-4	comment-4	Mock biopsy results					Gross pathology			Age at death							
		blo	gut-h	gut-z	node	node	sum-			hist-36	gut-comment-4	gut-l	gut-z	node	node	sum-zn-b	biops		gross	ti	mln	lymph: surgery site	fat re:		
8	02-6916	1	+	+++	+	+	+++	3b	severe diffuse multibacillary	severe multifocal to diffuse, occ AFB	necropsied Aug-02, no mock biopsy										+			24m	
10	03/6005		+	++	+	+	++	3c			necropsied Aug-03	+	++	+	+	++	3c	+	+	++	+	-		small	35m
13	03/1026	1	+	++	+	+	++	3b	severe diffuse multibacillary	severe multifocal	necropsied Feb-03	+	++	+	+	++	3b	+	+	++	+++	-		none	29m
22											died in paddock - not sampled Aug-02														24m, no necropsy
29	03/1026	2	+	+++	+	+++	+++	3b	severe diffuse multibacillary	mild multifocal, but +++ within these	necropsied Feb-03	+	+++	+	+++	+++	3b	+	+++	+	+++	-		none	29m
46	03/3703		+	+++	+	+	+++	3b			necropsied jun-03	+	+++	+	+	+++	3b	+	+	+	+	-			33m
53											died in paddock - not sampled Oct-02														25m, no necropsy
54	03/0111	54	+	+	+	-	+	3a/c	Severe multifocal, with diffuse mononuclear inflam but only mild villous changes, lymphangitis	multifocal, not enlarged	necropsied Jan-03, no mock biopsy samples							+	+	-	+				28m
55	02/8203	25	+	+++	+	+	+++	3b	severe diffuse multibacillary	multifocal, few AFB, faint	necropsied Aug-02, no mock biopsy							nr							24m
58	03/5228		+	+++	+	+	+++	3b			necropsied jul-03	+	+++	-	+++	+++	3b	+	+	+	+	+		none	34m
68											died in paddock - not sampled Oct-02														25m, no necropsy
74	02/0111	74	+	+++	+	++	+++	3b	severe diffuse multibacillary	multifocal, not enlarged	necropsied Jan-03, no mock biopsy samples							+	+++	-	+				28m
76	02-6916	2?	+	-	+	-	-	3c	diffuse lymphoid infiltration, with villous blunting, multifocal macrophage	moderate multifocal	necropsied Aug-02, no mock biopsy							+	+	+	+				24m
78	03/1026	3	+	+++	+	+++	+++	3b	severe diffuse multibacillary	severe multifocal	necropsied Feb-03	+	+++	+	+++	+++	3b	+	+	+	+++	-		none	29m

Appendix 2h. Culture results from tissues collected at biopsy, 12 mth (Sep 2001)

Sheep ID	Mesenteric lymph nodes			MLN-sum*	Terminal ileum		TI-sum*	Combined summary result*		
	conv	spun			conv	spun				
1	+	98	-	+	-	-	-	+		
2	-		-	-	-	-	-	-		
4	-		-	-	-	-	-	-		
5	-		-	-	-	-	-	-		
6	-		-	-	-	-	-	-		
7	-		+	41	+	-	-	+		
8	-		-	-	-	+	42	+		
10	-		-	-	-	-	-	-		
11	-		-	-	-	-	-	-		
12	-		-	-	-	-	-	-		
13	-		-	-	-	-	-	-		
14	-		-	-	-	-	-	-		
15	-		-	-	-	-	-	-		
16	-		-	-	-	-	-	-		
17	-		-	-	-	-	-	-		
18	-		-	-	-	-	-	-		
19	-		-	-	-	-	-	-		
20	-		-	-	-	-	-	-		
21	-		-	-	-	-	-	-		
22	-		-	-	-	-	-	-		
23	-		-	-	+	30	-	+		
24	-		-	-	-	-	-	-		
26	-		-	-	-	-	-	-		
27	+	32	+	38	+	-	-	+		
28	-		-	-	-	-	-	-		
29	-		-	-	-	-	-	-		
30	+	34	+	26	+	-	+	43	+	
31	-		-	-	-	-	-	-		
32	-		-	-	-	-	-	-		
33	-		-	-	-	-	-	-		
35	+	39	+	37	+	-	+	48	+	
36	-		-	-	-	-	-	-		
37	-		-	-	-	-	-	-		
38	-		-	-	-	-	-	-		
39	-		-	-	-	-	-	-		
40	-		-	-	-	-	-	-		
41	-		-	-	-	-	-	-		
42	-		-	-	-	-	-	-		
43	-		-	-	-	-	-	-		
44	-		-	-	-	-	-	-		
45	-		-	-	-	-	-	-		
46	-		-	-	-	-	-	-		
47	-		-	-	-	-	-	-		
49	-		-	-	-	-	-	-		
50	-		-	-	-	-	-	-		
51	-		-	-	-	-	-	-		
52	-		-	-	-	-	-	-		
53	-		-	-	-	-	-	-		
54	+	31	+	27	+	+	39	+	35	+
55	-		-	-	-	-	-	-	-	
56	-		-	-	-	-	-	-	-	
57	-		-	-	-	-	-	-	-	
58	+	44	+	38	+	-	-	-	-	+
59	-		-	-	-	-	-	-	-	
60	-		-	-	-	-	-	-	-	
61	-		-	-	-	-	-	-	-	
62	-		-	-	-	-	-	-	-	
63	+	75	+	42	+	-	-	-	-	+

Appendix 2h. Culture results from tissues collected at biopsy, 12 mth (Sep 2001)

Sheep ID	Mesenteric lymph nodes			Terminal ileum			TI-sum*	Combined summary result*
	conv	spun	MLN-sum*	conv	spun	TI-sum*		
64	-	-	-	-	-	-	-	-
65	+ 29	+ 24	+	-	-	-	-	+
66	-	-	-	-	-	-	-	-
67	-	-	-	-	-	-	-	-
68	-	-	-	-	-	-	-	-
69	-	-	-	-	-	-	-	-
70	+ 36	+ 36	+	-	-	-	-	+
71	-	-	-	-	-	-	-	-
72	-	-	-	-	+ 30	+	+	+
73	-	-	-	-	-	-	-	-
74	-	-	-	-	-	-	-	-
75	-	-	-	-	-	-	-	-
76	+ 30	+ 25	+	+ 30	+ 23	+	+	+
77	-	-	-	-	-	-	-	-
78	+ 30	+ 24	+	-	-	-	-	+
79	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-
81	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-

* Data are based on both spun and conventional tissue culture methods; positive if either positive

Appendix 2h. Culture results from tissues collected at biopsy, 18 mth (Mar 2002)

Sheep ID	Mesenteric lymph nodes			MLN-sum*	Terminal ileum			TI-sum*	Combined summary result*
	conv	spun			conv	spun			
1	-	-		-	-	-	-	-	
2									
4									
5									
6									
7	-	-		-	-	-	-	-	
8	+ 31	+ 31		+	+ 25	+ 10	+	+	
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23	-	-		-	-	-	-	-	
24									
26									
27	+ 35	+ 36		+	+ 38	+ 31	+	+	
28									
29									
30	-	-		-	+ 38	+ 37	+	+	
31	-	-		-	-	-	-	-	
32									
33									
35	-	-		-	-	-	-	-	
36									
37									
38									
39									
40									
41									
42									
43									
44									
45									
46									
47									
49									
50	+ 38	+ 36		+	+ 39	+ 33	+	+	
51	-	-		-	-	-	-	-	
52									
53									
54	+ 31	+ 29		+	+ 31	+ 29	+	+	
55									
56									
57									
58	-	-		-	-	-	-	-	
59									
60									
61									
62									
63	-	-		-	-	-	-	-	

Appendix 2h. Culture results from tissues collected at biopsy, 18 mth (Mar 2002)

Sheep ID	Mesenteric lymph nodes				Terminal ileum			TI-sum*	Combined summary result*
	conv	spun	MLN-sum*	conv	spun	TI-sum*			
64									
65	+ 50	+ 50	+	-	-	-	-	+	
66									
67									
68									
69									
70	-	-	-	-	-	-	-	-	
71									
72	-	-	-	-	-	-	-	-	
73	-	-	-	-	-	-	-	-	
74									
75									
76	+ 31	+ 30	+	+ 34	+ 26	+	+	+	
77	-	-	-	-	-	-	-	-	
78	+ 31	-	+	+ 33	+ 26	+	+	+	
79									
80									
81									
90									

* Data are based on both spun and conventional tissue culture methods; positive if either positive

Appendix 2h. Culture results from tissues collected at biopsy, - 24 mth (Sep 2002)

Sheep ID	Mesenteric lymph nodes				Terminal ileum			Combined summary result*
	conv	spun	MLN-sum*	conv	spun	TI-sum*		
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
4	c	c	c	c	c	c	c	contamination of batch of bactecs
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	c	c	c	c	c	c	c	contamination of batch of bactecs
8	+ 21	+ 17	+	+ 11	+ 9	+	+	necropsy data
10	+ 39	+ 37	+	+ 28	+ 28	+	+	
11	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
13	+ 35	+ 28	+	+ 17	+ 9	+	+	
14	c	-	-	c	c	c	-	contamination of batch of bactecs
15	+ 38	+ 31	+	+ 38	+ 32	+	+	
16	-	-	-	-	-	-	-	-
17	c	c	c	c	c	c	c	contamination of batch of bactecs
18	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-
21	c	-	-	c	c	c	-	contamination of batch of bactecs
22	-	-	-	-	-	-	-	died in paddack, no biopsy
23	-	-	-	-	-	-	-	-
24	c	-	-	c	c	c	-	contamination of batch of bactecs
26	c	c	c	-	c	-	-	contamination of batch of bactecs
27	-	-	-	-	+ 54	-	+	
28	-	-	-	-	-	-	-	-
29	+ 28	+ 25	+	+ 18	+ 11	+	+	
30	-	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-
32	c	c	c	c	c	c	c	contamination of batch of bactecs
33	-	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-	-
36	-	-	-	-	-	-	-	-
37	c	c	c	c	c	c	c	contamination of batch of bactecs
38	c	c	c	c	c	c	c	contamination of batch of bactecs
39	c	c	c	c	c	c	c	contamination of batch of bactecs
40	-	-	-	-	-	-	-	-
41	c	c	c	c	c	c	c	contamination of batch of bactecs
42	-	-	-	-	-	-	-	-
43	-	-	-	-	-	-	-	-
44	c	c	c	c	c	c	c	contamination of batch of bactecs
45	c	c	c	c	-	-	-	contamination of batch of bactecs
46	c	c	c	c	c	c	c	contamination of batch of bactecs
47	c	c	c	c	-	-	-	contamination of batch of bactecs
49	-	-	-	-	-	-	-	-
50	c	c	c	c	c	c	c	contamination of batch of bactecs
51	c	c	c	c	c	c	c	contamination of batch of bactecs
52	-	-	-	-	-	-	-	-
53	+ 37	+ 32	+	+ 18	+ 11	+	+	
54	+ 32	+ 27	+	+ 31	+ 24	+	+	
55	+ 28	+ 22	+	+ 11	+ 6	+	+	necropsy data, mock biopsy
56	-	-	-	-	-	-	-	-
57	-	-	-	-	-	-	-	-
58	+ 39	+ 35	+	+ 31	+ 25	+	+	
59	-	-	-	-	-	-	-	-
60	-	+ 39	+	-	+ 42	+	+	
61	-	-	-	-	-	-	-	-
62	-	-	-	-	-	-	-	-
63	-	-	-	-	-	-	-	-

Appendix 2h. Culture results from tissues collected at biopsy, - 24 mth (Sep 2002)

Sheep ID	Mesenteric lymph nodes			Terminal ileum			TI-sum*	Combined summary result*
	conv	spun	MLN-sum*	conv	spun	TI-sum*		
64	-	+ 37	+	-	-	-	-	+
65	-	-	-	-	-	-	-	-
66	-	-	-	-	-	-	-	-
67	-	-	-	-	-	-	-	-
68	-	-	-	-	-	-	-	-
69	c	-	-	-	c	-	-	contamination of batch of bactecs
70	-	-	-	-	-	-	-	-
71	c	c	c	c	c	c	c	contamination of batch of bactecs
72	-	-	-	-	-	-	-	-
73	-	-	-	-	-	-	-	-
74	+ 33	+ 30	+	+ 15	+ 10	+	+	+
75	-	-	-	-	-	-	-	-
76	+ 39	+ 31	+	+ 38	+ 32	+	+	necropsy data
77	-	-	-	-	-	-	-	-
78	+ 41	+ 26	+	+ 25	+ 18	+	+	+
79	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-
81	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-

* Data are based on both spun and conventional tissue culture methods; positive if either positive

Appendix 2h. Culture results from tissues collected at biopsy, - Sep 03, necropsy and mock biopsy samples

Sheep ID	Necropsy examination ^a					Mock biopsies ^b							Necropsy date						
	MLN		TI	ICV	Pool	Combined summary result	Mesenteric lymph nodes			Terminal ileum		Combined summary result							
	+	-	+	+	+	+	spun	conv	min sum	spun	conv	ti sum							
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
5	+	21	+	20	+	22	+	20	+	28	+	35	+	22	+	30	+	+	
6	-	-	-	-	+	34	+	37	+	-	-	-	-	-	-	-	-	-	
7	-	-	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
8	+	17	+	9	+	9	+	9	+	-	-	-	-	-	-	-	-	nd	Aug-02
10	+	27	+	23	c	c	+	c	+	31	+	46	+	27	+	39	+	+	Aug-03
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	26	+	9	+	15	+	12	+	23	+	28	+	12	+	22	+	+	Feb-03
14	+	38	-	-	-	+	44	+	+	62	-	-	+	-	-	-	-	+	-
15	c	-	c	-	+	22	+	22	+	41	c	-	+	30	c	-	+	+	-
16	+	30	-	-	-	+	40	+	+	36	-	-	+	-	-	-	-	+	-
17	+	26	+	22	+	22	+	22	+	30	+	37	+	26	+	35	+	+	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	+	50	-	-	-	+	61	+	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	nd	-	-	-	-	-	-	-	-	-	-	nd	dead in paddock
23	+	29	+	9	+	16	+	10	+	35	+	44	+	16	+	24	+	+	-
24	-	-	-	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	+	25	+	19	+	17	+	19	+	38	-	-	+	26	+	31	+	+	-
27	+	27	+	19	+	34	+	20	+	31	+	32	+	29	+	39	+	+	-
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	+	10	+	9	+	9	+	9	+	11	+	17	+	9	+	9	+	+	Feb 02
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	+	33	c	-	-	+	33	+	+	40	-	-	+	-	-	-	-	+	-
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	+	38	-	-	-	+	39	+	c	-	-	-	-	-	-	-	-	-	-
37	+	38	+	31	+	33	-	-	+	30	+	38	+	36	+	45	+	+	-
38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	+	33	-	-	-	+	34	+	+	38	+	40	+	-	-	-	-	+	-
40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	-	-	-	-	-	+	47	+	-	-	-	-	-	-	-	-	-	-	-
42	+	37	+	38	+	35	+	39	+	45	-	-	+	52	-	-	+	+	-
43	+	46	+	46	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
44	+	36	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	+	17	+	7	+	10	+	10	+	20	+	26	+	9	+	14	+	+	May 03
47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 2h. Culture results from tissues collected at biopsy, - Sep 03, necropsy and mock biopsy samples

Sheep ID	Necropsy examination ^a						Mock biopsies ^b						Necropsy date								
	MLN		TI		ICV		Pool		Combined summary result		Mesenteric lymph nodes			Terminal ileum		Combined summary result					
	+	-	+	-	+	-	+	-	+	-	spun	conv		min sum	spun	conv	ti sum	+	-		
49	+	29	+	12	+	12	+	12	+	+	+	38	+	70	+	+	13	+	17	+	+
50	+	35	+	29	+	30	+	34	+	+	+	37	+	56	+	+	36	+	43	+	+
51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	-	-	-	-	-	-	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-
53	-	-	-	-	-	-	-	-	nd	-	-	-	-	-	-	-	-	-	-	-	nd
54	nd	-	+	28	nd	-	nd	-	+	+	+	32	+	33	+	+	26	+	33	+	+
55	+	21	+	6	+	7	+	7	+	+	+	22	+	28	+	+	6	+	11	+	+
56	+	49	-	-	-	-	+	50	+	-	-	-	-	-	-	-	-	-	-	-	-
57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58	+	14	+	9	+	9	+	9	+	+	+	9	+	13	+	+	16	+	23	+	+
59	-	-	-	-	-	-	-	-	-	c	c	-	-	-	-	-	-	-	-	-	-
60	+	17	+	9	+	9	+	9	+	+	+	19	+	23	+	+	13	+	18	+	+
61	+	54	+	45	-	-	+	40	+	-	-	-	-	-	-	-	-	-	-	-	-
62	+	35	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
63	+	48	-	-	-	-	-	-	+	+	+	48	-	-	+	-	-	-	-	-	+
64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
65	-	-	-	-	+	51	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
66	+	34	-	-	c	-	+	35	+	-	-	-	-	-	c	-	-	-	-	-	-
67	+	35	-	-	-	-	+	39	+	-	-	-	-	-	-	-	-	-	-	-	-
68	-	-	-	-	-	-	-	-	nd	-	-	-	-	-	-	-	-	-	-	-	nd
69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70	+	35	-	-	-	-	+	40	+	+	+	38	-	-	+	-	-	-	-	-	+
71	+	29	+	14	+	22	+	15	+	+	+	41	+	43	+	+	36	+	44	+	+
72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
74	nd	-	+	13	nd	-	nd	-	+	+	+	15	+	18	+	+	13	+	13	+	+
75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
76	+	31	+	32	+	31	+	31	+	-	-	-	-	-	-	-	-	-	-	-	nd
77	+	38	-	-	-	-	+	43	+	-	-	-	-	-	-	-	-	-	-	-	-
78	+	17	+	9	+	10	+	10	+	+	+	18	+	25	+	+	10	+	13	+	+
79	+	39	+	38	+	29	+	30	+	-	-	-	-	-	-	-	-	-	-	-	-
80	+	26	+	33	+	37	+	27	+	+	+	33	+	37	+	+	43	-	-	+	+
81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	c	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

^a All cultures from necropsy performed using the spun protocol

^b Data are based on both spun and conventional tissue culture methods; positive if either positive

These sheep died or were euthanased prior to Sep 03

Appendix 3. Summary results at each sampling time for each sheep

Appendix 3a. Summary results of tests for humoral immunity: AGID and Elisa

Appendix 3b. Summary results of tests for cell mediated immunity: gamma interferon and skin testing for DTH

Appendix 3c. Summary of histopathology results: from biopsies and necropsy

Appendix 3d. Summary results for culture from tissues: biopsy and necropsy examinations

Appendix 3e. Summary of biopsy results: includes culture and/or histo positive

Appendix 3a. Summary results of tests for humoral immunity: AGID and Elisa

Sheep ID	AGID tests							Elisa tests					Humoral reactor	OJD status	Age at death
	9	12	18	24	30	36	sum	18	24	30	36	sum			
1	-	-	-	-	-	-	-	-	+	-	-	+	+	recovered	
2	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
4	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
5	-	-	-	-	-	-	-	-	-	+	-	+	+	mild OJD	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
7	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
8	-	-	-	+++	-	-	+	+	-	-	-	+	+	died OJD	24m
10	-	-	-	+	++	++	+	+	+	+	+	+	+	died OJD	35m
11	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
12	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
13	-	-	-	-	+++	-	+	-	-	+	-	+	+	died OJD	29m
14	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
15	-	-	-	-	-	-	-	-	-	-	-	-	-	severe OJD	
16	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
17	-	-	-	-	-	-	-	-	-	-	+	+	+	severe OJD	
18	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
19	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
20	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
21	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
22	-	-	-	-	-	-	-	-	-	-	-	-	-	dead, mild OJ	24m
23	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
24	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
26	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
27	-	-	-	-	-	-	-	-	-	-	+	+	+	mild OJD	
28	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
29	-	-	-	-	+++	-	+	-	+	+	-	+	+	died OJD	29m
30	-	-	+	-	+	-	+	+	+	-	-	+	+	recovered	
31	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
32	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
33	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
35	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
36	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
37	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
38	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
39	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
40	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
41	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
42	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
43	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
44	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
45	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
46	-	-	-	-	-	inc	-	-	-	+	+	+	+	died OJD	33m
47	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
49	-	-	-	-	-	-	+	+	-	-	-	+	+	severe OJD	
50	-	-	-	-	+	-	+	-	+	-	-	+	+	severe OJD	
51	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
52	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
53	-	-	-	-	-	-	-	-	-	-	-	-	-	dead, severe	25m
54	-	-	++	+++	++	-	+	+	+	-	-	+	+	died OJD	28m
55	-	-	-	+++	-	-	+	-	+	-	-	+	+	died OJD	24m
56	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
57	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
58	-	-	-	-	+	+++	+	-	-	-	+	+	+	died OJD	34m
59	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
60	-	-	-	-	-	+	+	-	-	-	-	-	+	severe OJD	
61	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
62	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
63	-	-	-	-	-	-	-	+	+	+	+	+	+	mild OJD	
64	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
65	-	-	-	-	-	-	-	+	+	+	-	+	+	mild OJD	
66	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
67	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
68	-	-	-	-	-	-	-	-	-	-	-	-	-	dead, uninfec	25m

Appendix 3a. Summary results of tests for humoral immunity: AGID and Elisa

Sheep ID	AGID tests							Elisa tests					Humoral reactor	OJD status	Age at death	
	9	12	18	24	30	36	sum	18	24	30	36	sum				
69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	severe OJD	
72	-	-	-	-	-	-	-	-	+	-	-	+	+	+	recovered	
73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
74	-	-	-	-	++	-	+	-	-	-	-	-	-	+	died OJD	28m
75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
76	-	-	-	-	-	-	-	-	+	-	-	-	+	+	died OJD	24m
77	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
78	-	-	-	-	+++	-	+	-	-	-	-	-	-	+	died OJD	29m
79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
Total +ve	0	0	2	2	4	2		6	9	5	4					
Total sheep	77	77	77	74	66	63		77	74	66	63					
% +ve	0.0	0.0	2.6	2.7	6.1	3.2		7.8	12.2	7.6	6.3					
For analysis:	0/74	0/74	2/74	2/71	4/66	2/63		6/74	9/71	5/66	4/63					

These sheep excluded from statistical analysis

Samples collected at necropsy. Not included in % positive, total sheep, nor in statistical analysis

Appendix 3b. Summary results of tests for cell mediated immunity: gamma interferon and skin testing fc

SheepID	Gamma interferon results							Skin testing results				CMI		Age at death
	9	12	18	24	30	36	Summ	24	30	36	Summ	Summ	OJD status	
1	-	-	-	+	+	-	+	+	-	-	+	+	recovered	
2	-	-	-	-	-	-	-	+	-	-	+	+	mild OJD	
4	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
5	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
6	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
7	-	-	-	-	-	-	-	+	+	-	+	+	recovered	
8	-	-	+	-	-	-	+	-	-	-	-	+	died OJD	24m
10	-	-	+	-	+	+	+	+	-	-	+	+	died OJD	35m
11	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
12	-	-	-	-	+	-	+	-	-	-	-	+	uninfected/r	
13	-	-	-	+	-	-	+	-	-	-	-	+	died OJD	29m
14	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
15	-	-	-	-	+	-	+	+	-	-	+	+	severe OJD	
16	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
17	-	-	-	-	+	-	+	-	-	+	+	+	severe OJD	
18	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
19	-	-	-	-	-	-	-	-	+	-	+	+	mild OJD	
20	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
21	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
22	-	-	+	-	-	-	+	-	-	-	-	+	dead, mild OJD	24m
23	-	-	-	-	+	+	+	-	+	-	+	+	mild OJD	
24	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
26	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
27	+	-	+	+	+	+	+	+	+	-	+	+	mild OJD	
28	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
29	+	-	+	-	-	-	+	+	-	-	+	+	died OJD	29m
30	-	-	+	-	+	+	+	+	-	-	+	+	recovered	
31	-	-	-	-	+	+	+	+	-	-	+	+	mild OJD	
32	-	-	-	-	+	+	+	+	+	-	+	+	uninfected/r	
33	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
35	-	-	-	-	+	-	+	+	-	-	+	+	mild OJD	
36	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
37	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
38	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
39	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
40	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
41	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
42	-	-	-	-	+	-	+	+	-	-	+	+	mild OJD	
43	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
44	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
45	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
46	-	-	-	-	+	-	+	+	+	-	+	+	died OJD	33m
47	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
49	-	-	-	-	+	+	+	+	-	-	+	+	severe OJD	
50	+	-	+	+	+	+	+	+	+	+	+	+	severe OJD	
51	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
52	-	-	-	-	+	+	+	-	-	-	-	+	recovered	
53	-	-	-	-	-	-	-	+	-	-	+	+	dead, severe O.	25m
54	-	-	+	-	-	-	+	-	-	-	-	+	died OJD	28m
55	-	-	-	-	-	-	-	-	-	-	-	-	died OJD	24m

Appendix 3b. Summary results of tests for cell mediated immunity: gamma interferon and skin testing for

SheepID	Gamma interferon results							Skin testing results				CMI		Age at death
	9	12	18	24	30	36	Summ	24	30	36	Summ	Summ	OJD status	
56	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
57	-	-	-	-	-	-	-	+	-	-	+	+	recovered	
58	-	-	-	+	+	+	+	+	-	-	+	+	died OJD	34m
59	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
60	-	-	-	-	+	-	+	-	-	-	-	+	severe OJD	
61	-	-	-	+	+	-	+	-	-	-	-	+	mild OJD	
62	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
63	+	-	-	-	+	-	+	+	-	-	+	+	mild OJD	
64	-	-	-	-	+	-	+	-	-	-	-	+	recovered	
65	-	-	-	-	-	-	-	+	+	-	+	+	mild OJD	
66	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
67	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
68	-	-	-	-	-	-	-	-	-	-	-	-	dead, uninfected	25m
69	-	-	-	-	+	-	+	-	-	-	-	+	uninfected/r	
70	-	-	-	-	-	+	+	-	-	-	-	+	mild OJD	
71	-	-	-	-	+	-	+	-	-	-	-	+	severe OJD	
72	+	-	-	-	+	-	+	+	-	-	+	+	recovered	
73	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
74	-	-	-	+	-	-	+	+	-	-	+	+	died OJD	28m
75	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
76	+	+	+	+	-	-	+	-	-	-	-	+	died OJD	24m
77	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
78	-	-	+	+	-	-	+	+	-	-	+	+	died OJD	29m
79	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
80	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
81	-	-	-	-	-	-	-	+	-	-	+	+	uninfected/r	
90	-	-	-	-	+	-	+	-	-	-	-	+	uninfected/r	
Total +ve	6	1	10	8	32	9		24	8	2.0				
Total sheep	77	77	77	74	66	63		74	66	63.0				
% +ve	7.8	1.3	13.0	10.8	48.5	14.3		32.4	12.1	3.2				
For analysis:	6/74	1/74	9/74	8/71	32/66	9/63		23/71	8/66	2/63				

These sheep excluded from statistical analysis

Samples collected at necropsy. Not included in % positive, total sheep, nor in statistical analysis

Appendix 3c. Summary of histopathology results: from biopsies and necropsy

Sheep ID	Findings from biopsy				Necropsy	Peak pathology	OJD status	Age at death
	12	18	24	36*				
1	-	-	-	?	-	-	recovered	
2	-	-	2	1	1	2	mild OJD	
4	-	-	-	-	-	-	recovered	
5	-	-	-	2	2	2	mild OJD	
6	?	-	-	?	?	-	mild OJD	
7	?	?	1	?	?	1	recovered	
8	?	3b	-	-	3b	3b	died OJD	24m
10	-	-	3c	3c	3c	3c	died OJD	35m
11	?	-	-	-	-	-	uninfected/n	
12	?	-	-	-	-	-	uninfected/r	
13	-	-	3b	3b	3b	3b	died OJD	29m
14	-	-	-	1	1	1	mild OJD	
15	?	-	3c	3c	3c	3c	severe OJD	
16	-	-	-	1	1	1	mild OJD	
17	-	-	-	3c	3c	3c	severe OJD	
18	-	-	-	-	-	-	uninfected/n	
19	?	-	-	1	1	1	mild OJD	
20	-	-	-	-	-	-	uninfected/n	
21	-	-	-	-	-	-	uninfected/n	
22	-	-	-	-	-	-	dead, mild OJ	24m
23	-	-	-	3a	3a	3a	mild OJD	
24	-	-	-	-	-	-	uninfected/n	
26	-	-	-	2	2	2	mild OJD	
27	?	3c	3a	3c	2	3a	mild OJD	
28	?	-	-	-	-	-	uninfected/n	
29	-	-	3b	3b	3b	3b	died OJD	29m
30	2n	3a	-	?	-	3a	recovered	
31	?	?	-	1	1	1	mild OJD	
32	-	-	-	-	-	-	uninfected/r	
33	-	-	-	-	-	-	uninfected/n	
35	-	?	1	-	1	1	mild OJD	
36	-	-	-	-	2(n)	2(n)	mild OJD	
37	-	-	-	2	2(n)	2(n)	mild OJD	
38	-	-	-	-	-	-	uninfected/n	
39	?	-	-	2(n)	?	2(n)	mild OJD	
40	-	-	?	?	?	-	recovered	
41	-	-	-	-	-	-	mild OJD	
42	?	-	-	-	-	-	mild OJD	
43	?	-	-	?	2(n)	2(n)	mild OJD	
44	-	-	-	-	?	-	mild OJD	
45	-	-	-	-	-	-	uninfected/n	
46	-	-	3a	3b	3b	3b	died OJD	33m
47	-	-	-	1	?	1	mild OJD	
49	-	-	-	3b	3b/3c	3b/3c	severe OJD	
50	-	3c	3c	3c	3c	3c	severe OJD	
51	-	1	-	-	-	1	recovered	
52	-	-	1	-	-	1	recovered	
53	?	-	3b	-	-	3b	dead, severe	25m
54	2	3c	3c	-	3a/c	3c	died OJD	28m
55	-	-	-	-	3b	3b	died OJD	24m
56	-	-	-	?	2(n)	2(n)	mild OJD	
57	-	-	1	?	?	1	recovered	
58	-	-	3c	3b	3b	3b	died OJD	34m
59	-	-	-	-	-	-	uninfected/n	

Appendix 3c. Summary of histopathology results: from biopsies and necropsy

Sheep ID	Findings from biopsy				Necropsy	Peak pathology	OJD status	Age at death
	12	18	24	36*				
60	?		-	3a	3b/3c	3b/3c	severe OJD	
61	-		-	1	1	1	mild OJD	
62	-		-	-	-	-	mild OJD	
63	1	-	-	?	?	1	mild OJD	
64	-		-	-	-	-	recovered	
65	-	-	?	?	?	-	mild OJD	
66	-		-	-	-	-	mild OJD	
67	-		-	?	?	-	mild OJD	
68	-		-	-	-	-	dead, uninfec	25m
69	-		-	-	-	-	uninfected/r	
70	-	?	-	?	-	-	mild OJD	
71	-		-	3a/3c	3b/3c	3b/3c	severe OJD	
72	-	?	-	?	?	-	recovered	
73	-	-	-	-	-	-	recovered	
74	?		3b	-	3b	3b	died OJD	28m
75	-		-	-	-	-	uninfected/n	
76	2	3c		-	3c	3c	died OJD	24m
77	-		-	-	-	-	mild OJD	
78	-	2n	3b/3c	3b	3b	3b	died OJD	29m
79	-		-	-	-	-	mild OJD	
80	-		-	2(n)	2	2	mild OJD	
81	-		-	-	?	-	uninfected/r	
90	-		-	-	-	-	uninfected/r	
Total +ve	4	8	17	20	22			
Total sheep	77	20	73	63	63			
% +ve	5.2	40.0	23.3	31.7	34.9			
For analysis:	4/74		16/71	20/63	22/63			

These sheep excluded from statistical analysis

Samples collected at early necropsy. Not included in % positive, total sheep, nor in statistical analysis

* These are mock biopsy samples collected during necropsy examination

Appendix 3d. Summary results for culture from tissues: biopsy and necropsy examinations

Sheep ID	Biopsy examinations									Necropsy					Final OJD sta			
	12 months			18 months			24 months			36 months*			Age at death					
	mln	ti	sum	mln	ti	sum	mln	ti	sum	mln	ti	sum	mln	ti		icv	pool	sum
1	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD
4	-	-	-	-	-	-	C	C	C	-	-	-	-	-	-	-	-	recovered
5	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	mild OJD
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	mild OJD
7	+	-	+	-	-	-	C	C	C	-	-	-	-	C	-	-	-	recovered
8	-	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	24m died OJD
10	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	C	+	35m died OJD
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r
13	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	29m died OJD
14	-	-	-	-	-	-	-	C	-	+	-	+	+	-	-	+	+	mild OJD
15	-	-	-	-	-	-	+	+	+	+	+	+	C	C	+	+	+	severe OJD
16	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+	mild OJD
17	-	-	-	-	-	-	C	C	C	+	+	+	+	+	+	+	+	severe OJD
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n
19	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	mild OJD
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n
21	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	uninfected/n
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	nd 24m dead, mild C
23	-	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	mild OJD
24	-	-	-	-	-	-	-	C	-	-	-	-	-	C	-	-	-	uninfected/n
26	-	-	-	-	-	-	C	-	-	+	+	+	+	+	+	+	+	mild OJD
27	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	mild OJD
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n
29	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	29m died OJD
30	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	recovered
31	-	-	-	-	-	-	-	-	-	+	-	+	+	C	-	+	+	mild OJD
32	-	-	-	-	-	-	C	C	C	-	-	-	-	-	-	-	-	uninfected/r
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n
35	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD
36	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	mild OJD
37	-	-	-	-	-	-	C	C	C	+	+	+	+	+	+	-	+	mild OJD
38	-	-	-	-	-	-	C	C	C	-	-	-	-	-	-	-	-	uninfected/n
39	-	-	-	-	-	-	C	C	C	+	-	+	+	-	-	+	+	mild OJD
40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered
41	-	-	-	-	-	-	C	C	C	-	-	-	-	-	-	+	+	mild OJD
42	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	mild OJD
43	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	mild OJD
44	-	-	-	-	-	-	C	C	C	-	-	-	-	+	-	-	-	mild OJD
45	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	uninfected/n
46	-	-	-	-	-	-	C	C	C	+	+	+	+	+	+	+	+	33m died OJD
47	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	mild OJD
49	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	severe OJD
50	-	-	-	+	+	+	C	C	C	+	+	+	+	+	+	+	+	severe OJD
51	-	-	-	-	-	-	C	C	C	-	-	-	-	-	-	-	-	recovered
52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	recovered
53	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	nd 25m dead, severe
54	+	+	+	+	+	+	+	+	+	+	+	+	nd	+	nd	nd	+	28m died OJD
55	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	24m died OJD
56	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	mild OJD
57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered
58	+	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	34m died OJD
59	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	uninfected/n

Appendix 3d. Summary results for culture from tissues: biopsy and necropsy examinations

Sheep ID	Biopsy examinations												Necropsy			Final OJD sta	
	12 months			18 months			24 months			36 months*			Age at death				
	mln	ti	sum	mln	ti	sum	mln	ti	sum	mln	ti	sum	mln	ti	icv	poolsum	
60	-	-	-				+	+	+	+	+	+	+	+	+	+	severe OJD
61	-	-	-				-	-	-	-	-	-	+	+	-	+	mild OJD
62	-	-	-				-	-	-	-	-	-	+	-	-	-	mild OJD
63	+	-	+	-	-	-	-	-	-	+	-	+	+	-	-	-	mild OJD
64	-	-	-				+	-	+	-	-	-	-	-	-	-	recovered
65	+	-	+	+	-	+	-	-	-	-	-	-	-	-	+	-	mild OJD
66	-	-	-				-	-	-	-	-	-	+	-	C	+	mild OJD
67	-	-	-				-	-	-	-	-	-	+	-	-	+	mild OJD
68	-	-	-				-	-	-								nd 25m dead, uninfected
69	-	-	-				-	-	-	-	-	-	-	-	-	-	uninfected/r
70	+	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	mild OJD
71	-	-	-				C	C	C	+	+	+	+	+	+	+	severe OJD
72	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered
73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered
74	-	-	-				+	+	+				nd	+	nd	nd	28m died OJD
75	-	-	-				-	-	-	-	-	-	-	-	-	-	uninfected/n
76	+	+	+	+	+	+							+	+	+	+	24m died OJD
77	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	mild OJD
78	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	29m died OJD
79	-	-	-				-	-	-	-	-	-	+	+	+	+	mild OJD
80	-	-	-				-	-	-	+	+	+	+	+	+	+	mild OJD
81	-	-	-				-	-	-	-	-	-	-	-	-	-	uninfected/r
90	-	-	-				-	-	-	-	-	-	-	-	-	-	uninfected/r
Total +ve	12	7	15	7	7	8	11	11	12				19				33
Total sheep			77			20			60				63				63
% +ve			19			40			20				30				52
For analysis:			15/74						11/57				19/63				33/63

These sheep excluded from statistical analysis

Samples collected at early necropsy. Not included in % positive, total sheep, nor in statistical analysis

* These are mock biopsy samples collected during necropsy examination

Appendix 3e. Summary of biopsy results: includes culture and/or histo positive

Sheep ID	Find Findings from biopsy												Necropsy		OJD status	Age at death	
	c	h	12	c	h	18	c	h	24	c	h	36*	c	h			
1	+	-	+	-	-	-	-	-	-	?	-	-	-	-	recovered		
2	-	-	-	-	-	-	-	2	+	-	1	+	-	1	+	mild OJD	
4	-	-	-	-	-	-	c	-	-	-	-	-	-	-	-	recovered	
5	-	-	-	-	-	-	-	-	-	+	2	+	+	2	+	mild OJD	
6	-	?	-	-	-	-	-	-	-	-	?	-	+	?	+	mild OJD	
7	+	?	+	-	?	-	c	1	+	-	?	-	-	?	-	recovered	
8	+	?	+	+	3b	+	-	-	-	-	-	-	+	3b	+	died OJD	24m
10	-	-	-	-	-	-	+	3c	+	+	3c	+	+	3c	+	died OJD	35m
11	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
12	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
13	-	-	-	-	-	-	+	3b	+	+	3b	+	+	3b	+	died OJD	29m
14	-	-	-	-	-	-	-	-	-	+	1	+	+	1	+	mild OJD	
15	-	?	-	-	-	-	+	3c	+	+	3c	+	+	3c	+	severe OJD	
16	-	-	-	-	-	-	-	-	-	+	1	+	+	1	+	mild OJD	
17	-	-	-	-	-	-	c	-	-	+	3c	+	+	3c	+	severe OJD	
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
19	-	?	-	-	-	-	-	-	-	-	1	+	+	1	+	mild OJD	
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
22	-	-	-	-	-	-	-	-	-	-	-	-	nd	-	-	dead, mild C	24m
23	+	-	+	-	-	-	-	-	-	+	3a	+	+	3a	+	mild OJD	
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
26	-	-	-	-	-	-	-	-	-	+	2	+	+	2	+	mild OJD	
27	+	?	+	+	3c	+	+	3a	+	+	3c	+	+	2	+	mild OJD	
28	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
29	-	-	-	-	-	-	+	3b	+	+	3b	+	+	3b	+	died OJD	29m
30	+	2n	+	+	3a	+	-	-	-	-	?	-	-	-	-	recovered	
31	-	?	-	-	?	-	-	-	-	+	1	+	+	1	+	mild OJD	
32	-	-	-	-	-	-	c	-	-	-	-	-	-	-	-	uninfected/r	
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
35	+	-	+	-	?	-	-	1	+	-	-	-	-	1	+	mild OJD	
36	-	-	-	-	-	-	-	-	-	-	-	-	+	2(n)	+	mild OJD	
37	-	-	-	-	-	-	c	-	-	+	2	+	+	2(n)	+	mild OJD	
38	-	-	-	-	-	-	c	-	-	-	-	-	-	-	-	uninfected/r	
39	-	?	-	-	-	-	c	-	-	+	2(n)	+	+	?	+	mild OJD	
40	-	-	-	-	-	-	-	?	-	-	?	-	-	?	-	recovered	
41	-	-	-	-	-	-	c	-	-	-	-	-	+	-	+	mild OJD	
42	-	?	-	-	-	-	-	-	-	+	-	+	+	-	+	mild OJD	
43	-	?	-	-	-	-	-	-	-	-	?	-	+	2(n)	+	mild OJD	
44	-	-	-	-	-	-	c	-	-	-	-	-	+	?	+	mild OJD	
45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
46	-	-	-	-	-	-	c	3a	+	+	3b	+	+	3b	+	died OJD	33m
47	-	-	-	-	-	-	-	-	-	-	1	+	-	-	+	mild OJD	
49	-	-	-	-	-	-	-	-	-	+	3b	+	+	3b/3c	+	severe OJD	
50	-	-	-	+	3c	+	c	3c	+	+	3c	+	+	3c	+	severe OJD	
51	-	-	-	-	1	+	c	-	-	-	-	-	-	-	-	recovered	
52	-	-	-	-	-	-	-	1	+	-	-	-	-	-	-	recovered	
53	-	?	-	-	-	-	+	3b	+	-	-	-	nd	-	-	dead, sever	25m
54	+	2	+	+	3c	+	+	3c	+	+		+	+	3a/c	+	died OJD	28m
55	-	-	-	-	-	-	-	-	-	-	-	-	+	3b	+	died OJD	24m
56	-	-	-	-	-	-	-	-	-	-	?	-	+	2(n)	+	mild OJD	
57	-	-	-	-	-	-	-	1	+	-	?	-	-	?	-	recovered	
58	+	-	+	-	-	-	+	3c	+	+	3b	+	+	3b	+	died OJD	34m
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	

Appendix 3e. Summary of biopsy results: includes culture and/or histo positive

Sheep ID	Find Findings from biopsy												Necropsy		OJD status	Age at death	
	c	h	12	c	h	18	c	h	24	c	h	36*	c	h			
60	-	?	-				+	-	+	+	3a	+	+	3b/3c	+	severe OJD	
61	-	-	-				-	-	-	-	1	+	+	1	+	mild OJD	
62	-	-	-				-	-	-	-	-	-	+	-	+	mild OJD	
63	+	1	+	-	-	-	-	-	-	+	?	+	+	?	+	mild OJD	
64	-	-	-				+	-	+	-	-	-	-	-	-	recovered	
65	+	-	+	+	-	+	-	?	-	-	?	-	+	?	+	mild OJD	
66	-	-	-				-	-	-	-	-	-	+	-	+	mild OJD	
67	-	-	-				-	-	-	-	?	-	+	?	+	mild OJD	
68	-	-	-				-	-	-				nd			dead, uninfected	25m
69	-	-	-				-	-	-	-	-	-	-	-	-	uninfected/r	
70	+	-	+	-	?	-	-	-	-	+	?	+	+	-	+	mild OJD	
71	-	-	-				c	-	-	+	3a/3c	+	+	3b/3c	+	severe OJD	
72	+	-	+	-	?	-	-	-	-	-	?	-	-	?	-	recovered	
73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
74	-	?	-				+	3b	+				+	3b	+	died OJD	28m
75	-	-	-				-	-	-	-	-	-	-	-	-	uninfected/r	
76	+	2	+	+	3c	+							+	3c	+	died OJD	24m
77	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	mild OJD	
78	+	-	+	+	2n	+	+	3b/3c	+	+	3b	+	+	3b	+	died OJD	29m
79	-	-	-				-	-	-	-	-	-	+	-	+	mild OJD	
80	-	-	-				-	-	-	+	2(n)	+	+	2	+	mild OJD	
81	-	-	-				-	-	-	-	-	-	-	?	-	uninfected/r	
90	-	-	-				-	-	-	-	-	-	-	-	-	uninfected/r	
Total +ve	15	4	15	8	8	9	12	17	19	19	20	23	33	22	36		
Total sheep	77	77	77	20	20	20	60	73	73	63	63	63	63	63	63		
% +ve	19	5.2	19.5	40	40.0	45.0	20	23.3	26.0	30.2	31.7	36.5	52.4	34.9	57.1		
For analysis:	15/74	4/74	15/74				11/58	16/71	18/71	19/63	20/63	23/63					

These sheep excluded from statistical analysis

Samples collected at early necropsy. Not included in % positive, total sheep, nor in statistical analysis

* These are mock biopsy samples collected during necropsy examination

** This sheep negative in the main necropsy histo, but positive in the mock biopsy

Appendix 3b. Summary results of tests for cell mediated immunity: gamma interferon and skin testing fc

SheepID	Gamma interferon results							Skin testing results				CMI		Age at death
	9	12	18	24	30	36	Summ	24	30	36	Summ	Summ	OJD status	
1	-	-	-	+	+	-	+	+	-	-	+	+	recovered	
2	-	-	-	-	-	-	-	+	-	-	+	+	mild OJD	
4	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
5	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
6	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
7	-	-	-	-	-	-	-	+	+	-	+	+	recovered	
8	-	-	+	-	-	-	+	-	-	-	-	+	died OJD	24m
10	-	-	+	-	+	+	+	+	-	-	+	+	died OJD	35m
11	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
12	-	-	-	-	+	-	+	-	-	-	-	+	uninfected/r	
13	-	-	-	+	-	-	+	-	-	-	-	+	died OJD	29m
14	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
15	-	-	-	-	+	-	+	+	-	-	+	+	severe OJD	
16	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
17	-	-	-	-	+	-	+	-	-	+	+	+	severe OJD	
18	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
19	-	-	-	-	-	-	-	-	+	-	+	+	mild OJD	
20	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
21	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
22	-	-	+	-	-	-	+	-	-	-	-	+	dead, mild OJD	24m
23	-	-	-	-	+	+	+	-	+	-	+	+	mild OJD	
24	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
26	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
27	+	-	+	+	+	+	+	+	+	-	+	+	mild OJD	
28	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
29	+	-	+	-	-	-	+	+	-	-	+	+	died OJD	29m
30	-	-	+	-	+	+	+	+	-	-	+	+	recovered	
31	-	-	-	-	+	+	+	+	-	-	+	+	mild OJD	
32	-	-	-	-	+	+	+	+	+	-	+	+	uninfected/r	
33	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
35	-	-	-	-	+	-	+	+	-	-	+	+	mild OJD	
36	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
37	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
38	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
39	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
40	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
41	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
42	-	-	-	-	+	-	+	+	-	-	+	+	mild OJD	
43	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
44	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
45	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
46	-	-	-	-	+	-	+	+	+	-	+	+	died OJD	33m
47	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
49	-	-	-	-	+	+	+	+	-	-	+	+	severe OJD	
50	+	-	+	+	+	+	+	+	+	+	+	+	severe OJD	
51	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
52	-	-	-	-	+	+	+	-	-	-	-	+	recovered	
53	-	-	-	-	-	-	-	+	-	-	+	+	dead, severe O.	25m
54	-	-	+	-	-	-	+	-	-	-	-	+	died OJD	28m
55	-	-	-	-	-	-	-	-	-	-	-	-	died OJD	24m

Appendix 3b. Summary results of tests for cell mediated immunity: gamma interferon and skin testing for

SheepID	Gamma interferon results							Skin testing results				CMI		Age at death
	9	12	18	24	30	36	Summ	24	30	36	Summ	Summ	OJD status	
56	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
57	-	-	-	-	-	-	-	+	-	-	+	+	recovered	
58	-	-	-	+	+	+	+	+	-	-	+	+	died OJD	34m
59	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
60	-	-	-	-	+	-	+	-	-	-	-	+	severe OJD	
61	-	-	-	+	+	-	+	-	-	-	-	+	mild OJD	
62	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
63	+	-	-	-	+	-	+	+	-	-	+	+	mild OJD	
64	-	-	-	-	+	-	+	-	-	-	-	+	recovered	
65	-	-	-	-	-	-	-	+	+	-	+	+	mild OJD	
66	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
67	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
68	-	-	-	-	-	-	-	-	-	-	-	-	dead, uninfected	25m
69	-	-	-	-	+	-	+	-	-	-	-	+	uninfected/r	
70	-	-	-	-	-	+	+	-	-	-	-	+	mild OJD	
71	-	-	-	-	+	-	+	-	-	-	-	+	severe OJD	
72	+	-	-	-	+	-	+	+	-	-	+	+	recovered	
73	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
74	-	-	-	+	-	-	+	+	-	-	+	+	died OJD	28m
75	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n	
76	+	+	+	+	-	-	+	-	-	-	-	+	died OJD	24m
77	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
78	-	-	+	+	-	-	+	+	-	-	+	+	died OJD	29m
79	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD	
80	-	-	-	-	+	-	+	-	-	-	-	+	mild OJD	
81	-	-	-	-	-	-	-	+	-	-	+	+	uninfected/r	
90	-	-	-	-	+	-	+	-	-	-	-	+	uninfected/r	
Total +ve	6	1	10	8	32	9		24	8	2.0				
Total sheep	77	77	77	74	66	63		74	66	63.0				
% +ve	7.8	1.3	13.0	10.8	48.5	14.3		32.4	12.1	3.2				
For analysis:	6/74	1/74	9/74	8/71	32/66	9/63		23/71	8/66	2/63				

These sheep excluded from statistical analysis

Samples collected at necropsy. Not included in % positive, total sheep, nor in statistical analysis

Appendix 3c. Summary of histopathology results: from biopsies and necropsy

Sheep ID	Findings from biopsy				Necropsy	Peak pathology	OJD status	Age at death
	12	18	24	36*				
1	-	-	-	?	-	-	recovered	
2	-	-	2	1	1	2	mild OJD	
4	-	-	-	-	-	-	recovered	
5	-	-	-	2	2	2	mild OJD	
6	?	-	-	?	?	-	mild OJD	
7	?	?	1	?	?	1	recovered	
8	?	3b	-	-	3b	3b	died OJD	24m
10	-	-	3c	3c	3c	3c	died OJD	35m
11	?	-	-	-	-	-	uninfected/n	
12	?	-	-	-	-	-	uninfected/r	
13	-	-	3b	3b	3b	3b	died OJD	29m
14	-	-	-	1	1	1	mild OJD	
15	?	-	3c	3c	3c	3c	severe OJD	
16	-	-	-	1	1	1	mild OJD	
17	-	-	-	3c	3c	3c	severe OJD	
18	-	-	-	-	-	-	uninfected/n	
19	?	-	-	1	1	1	mild OJD	
20	-	-	-	-	-	-	uninfected/n	
21	-	-	-	-	-	-	uninfected/n	
22	-	-	-	-	-	-	dead, mild OJ	24m
23	-	-	-	3a	3a	3a	mild OJD	
24	-	-	-	-	-	-	uninfected/n	
26	-	-	-	2	2	2	mild OJD	
27	?	3c	3a	3c	2	3a	mild OJD	
28	?	-	-	-	-	-	uninfected/n	
29	-	-	3b	3b	3b	3b	died OJD	29m
30	2n	3a	-	?	-	3a	recovered	
31	?	?	-	1	1	1	mild OJD	
32	-	-	-	-	-	-	uninfected/r	
33	-	-	-	-	-	-	uninfected/n	
35	-	?	1	-	1	1	mild OJD	
36	-	-	-	-	2(n)	2(n)	mild OJD	
37	-	-	-	2	2(n)	2(n)	mild OJD	
38	-	-	-	-	-	-	uninfected/n	
39	?	-	-	2(n)	?	2(n)	mild OJD	
40	-	-	?	?	?	-	recovered	
41	-	-	-	-	-	-	mild OJD	
42	?	-	-	-	-	-	mild OJD	
43	?	-	-	?	2(n)	2(n)	mild OJD	
44	-	-	-	-	?	-	mild OJD	
45	-	-	-	-	-	-	uninfected/n	
46	-	-	3a	3b	3b	3b	died OJD	33m
47	-	-	-	1	?	1	mild OJD	
49	-	-	-	3b	3b/3c	3b/3c	severe OJD	
50	-	3c	3c	3c	3c	3c	severe OJD	
51	-	1	-	-	-	1	recovered	
52	-	-	1	-	-	1	recovered	
53	?	-	3b	-	-	3b	dead, severe	25m
54	2	3c	3c	-	3a/c	3c	died OJD	28m
55	-	-	-	-	3b	3b	died OJD	24m
56	-	-	-	?	2(n)	2(n)	mild OJD	
57	-	-	1	?	?	1	recovered	
58	-	-	3c	3b	3b	3b	died OJD	34m
59	-	-	-	-	-	-	uninfected/n	

Appendix 3c. Summary of histopathology results: from biopsies and necropsy

Sheep ID	Findings from biopsy				Necropsy	Peak pathology	OJD status	Age at death
	12	18	24	36*				
60	?		-	3a	3b/3c	3b/3c	severe OJD	
61	-		-	1	1	1	mild OJD	
62	-		-	-	-	-	mild OJD	
63	1	-	-	?	?	1	mild OJD	
64	-		-	-	-	-	recovered	
65	-	-	?	?	?	-	mild OJD	
66	-		-	-	-	-	mild OJD	
67	-		-	?	?	-	mild OJD	
68	-		-	-	-	-	dead, uninfec	25m
69	-		-	-	-	-	uninfected/r	
70	-	?	-	?	-	-	mild OJD	
71	-		-	3a/3c	3b/3c	3b/3c	severe OJD	
72	-	?	-	?	?	-	recovered	
73	-	-	-	-	-	-	recovered	
74	?		3b	-	3b	3b	died OJD	28m
75	-		-	-	-	-	uninfected/n	
76	2	3c		-	3c	3c	died OJD	24m
77	-		-	-	-	-	mild OJD	
78	-	2n	3b/3c	3b	3b	3b	died OJD	29m
79	-		-	-	-	-	mild OJD	
80	-		-	2(n)	2	2	mild OJD	
81	-		-	-	?	-	uninfected/r	
90	-		-	-	-	-	uninfected/r	
Total +ve	4	8	17	20	22			
Total sheep	77	20	73	63	63			
% +ve	5.2	40.0	23.3	31.7	34.9			
For analysis:	4/74		16/71	20/63	22/63			

These sheep excluded from statistical analysis

Samples collected at early necropsy. Not included in % positive, total sheep, nor in statistical analysis

* These are mock biopsy samples collected during necropsy examination

Appendix 3d. Summary results for culture from tissues: biopsy and necropsy examinations

Sheep ID	Biopsy examinations									Necropsy					Final OJD sta			
	12 months			18 months			24 months			36 months*			Age at death					
	mln	ti	sum	mln	ti	sum	mln	ti	sum	mln	ti	sum	mln	ti	icv	poolsum		
1	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD
4	-	-	-	-	-	-	C	C	C	-	-	-	-	-	-	-	-	recovered
5	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	mild OJD
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	mild OJD
7	+	-	+	-	-	-	C	C	C	-	-	-	-	C	-	-	-	recovered
8	-	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	24m died OJD
10	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	C	+	35m died OJD
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r
13	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	29m died OJD
14	-	-	-	-	-	-	-	C	-	+	-	+	+	-	-	+	+	mild OJD
15	-	-	-	-	-	-	+	+	+	+	+	+	C	C	+	+	+	severe OJD
16	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+	mild OJD
17	-	-	-	-	-	-	C	C	C	+	+	+	+	+	+	+	+	severe OJD
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n
21	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	uninfected/n
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	nd 24m dead, mild C
23	-	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	mild OJD
24	-	-	-	-	-	-	-	C	-	-	-	-	-	C	-	-	-	uninfected/n
26	-	-	-	-	-	-	C	-	-	+	+	+	+	+	+	+	+	mild OJD
27	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	mild OJD
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n
29	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	29m died OJD
30	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	recovered
31	-	-	-	-	-	-	-	-	-	+	-	+	+	C	-	+	+	mild OJD
32	-	-	-	-	-	-	C	C	C	-	-	-	-	-	-	-	-	uninfected/r
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/n
35	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mild OJD
36	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	mild OJD
37	-	-	-	-	-	-	C	C	C	+	+	+	+	+	+	-	+	mild OJD
38	-	-	-	-	-	-	C	C	C	-	-	-	-	-	-	-	-	uninfected/n
39	-	-	-	-	-	-	C	C	C	+	-	+	+	-	-	+	+	mild OJD
40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered
41	-	-	-	-	-	-	C	C	C	-	-	-	-	-	-	+	+	mild OJD
42	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	mild OJD
43	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	mild OJD
44	-	-	-	-	-	-	C	C	C	-	-	-	+	-	-	-	+	mild OJD
45	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	uninfected/n
46	-	-	-	-	-	-	C	C	C	+	+	+	+	+	+	+	+	33m died OJD
47	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	mild OJD
49	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	severe OJD
50	-	-	-	+	+	+	C	C	C	+	+	+	+	+	+	+	+	severe OJD
51	-	-	-	-	-	-	C	C	C	-	-	-	-	-	-	-	-	recovered
52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	recovered
53	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	nd 25m dead, severe
54	+	+	+	+	+	+	+	+	+	+	+	+	nd	+	nd	nd	+	28m died OJD
55	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	24m died OJD
56	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	mild OJD
57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered
58	+	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	34m died OJD
59	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	uninfected/n

Appendix 3d. Summary results for culture from tissues: biopsy and necropsy examinations

Sheep ID	Biopsy examinations												Necropsy			Final OJD sta		
	12 months			18 months			24 months			36 months*			Age at death					
	mln	ti	sum	mln	ti	sum	mln	ti	sum	mln	ti	sum	mln	ti	icv	poolsum		
60	-	-	-				+	+	+	+	+	+	+	+	+	+	severe OJD	
61	-	-	-				-	-	-	-	-	-	+	+	-	+	mild OJD	
62	-	-	-				-	-	-	-	-	-	+	-	-	-	mild OJD	
63	+	-	+	-	-	-	-	-	-	+	-	+	+	-	-	-	mild OJD	
64	-	-	-				+	-	+	-	-	-	-	-	-	-	recovered	
65	+	-	+	+	-	+	-	-	-	-	-	-	-	-	+	-	mild OJD	
66	-	-	-				-	-	-	-	-	-	+	-	C	+	mild OJD	
67	-	-	-				-	-	-	-	-	-	+	-	-	+	mild OJD	
68	-	-	-				-	-	-								nd 25m dead, uninfected	
69	-	-	-				-	-	-	-	-	-	-	-	-	-	uninfected/r	
70	+	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	mild OJD	
71	-	-	-				C	C	C	+	+	+	+	+	+	+	severe OJD	
72	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
74	-	-	-				+	+	+				nd	+	nd	nd	+	28m died OJD
75	-	-	-				-	-	-	-	-	-	-	-	-	-	-	uninfected/n
76	+	+	+	+	+	+							+	+	+	+	+	24m died OJD
77	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	mild OJD
78	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	29m died OJD
79	-	-	-				-	-	-	-	-	-	+	+	+	+	+	mild OJD
80	-	-	-				-	-	-	+	+	+	+	+	+	+	+	mild OJD
81	-	-	-				-	-	-	-	-	-	-	-	-	-	-	uninfected/r
90	-	-	-				-	-	-	-	-	-	-	-	-	-	-	uninfected/r
Total +ve	12	7	15	7	7	8	11	11	12				19				33	
Total sheep			77			20			60				63				63	
% +ve			19			40			20				30				52	
For analysis:			15/74						11/57				19/63				33/63	

These sheep excluded from statistical analysis

Samples collected at early necropsy. Not included in % positive, total sheep, nor in statistical analysis

* These are mock biopsy samples collected during necropsy examination

Appendix 3e. Summary of biopsy results: includes culture and/or histo positive

Sheep ID	Find Findings from biopsy												Necropsy		OJD status	Age at death	
	c	h	12	c	h	18	c	h	24	c	h	36*	c	h			
1	+	-	+	-	-	-	-	-	-	?	-	-	-	-	recovered		
2	-	-	-	-	-	-	-	2	+	-	1	+	-	1	+	mild OJD	
4	-	-	-	-	-	-	c	-	-	-	-	-	-	-	-	recovered	
5	-	-	-	-	-	-	-	-	-	+	2	+	+	2	+	mild OJD	
6	-	?	-	-	-	-	-	-	-	-	?	-	+	?	+	mild OJD	
7	+	?	+	-	?	-	c	1	+	-	?	-	-	?	-	recovered	
8	+	?	+	+	3b	+	-	-	-	-	-	-	+	3b	+	died OJD	24m
10	-	-	-	-	-	-	+	3c	+	+	3c	+	+	3c	+	died OJD	35m
11	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
12	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
13	-	-	-	-	-	-	+	3b	+	+	3b	+	+	3b	+	died OJD	29m
14	-	-	-	-	-	-	-	-	-	+	1	+	+	1	+	mild OJD	
15	-	?	-	-	-	-	+	3c	+	+	3c	+	+	3c	+	severe OJD	
16	-	-	-	-	-	-	-	-	-	+	1	+	+	1	+	mild OJD	
17	-	-	-	-	-	-	c	-	-	+	3c	+	+	3c	+	severe OJD	
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
19	-	?	-	-	-	-	-	-	-	-	1	+	+	1	+	mild OJD	
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
22	-	-	-	-	-	-	-	-	-	-	-	-	nd	-	-	dead, mild C	24m
23	+	-	+	-	-	-	-	-	-	+	3a	+	+	3a	+	mild OJD	
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
26	-	-	-	-	-	-	-	-	-	+	2	+	+	2	+	mild OJD	
27	+	?	+	+	3c	+	+	3a	+	+	3c	+	+	2	+	mild OJD	
28	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
29	-	-	-	-	-	-	+	3b	+	+	3b	+	+	3b	+	died OJD	29m
30	+	2n	+	+	3a	+	-	-	-	-	?	-	-	-	-	recovered	
31	-	?	-	-	?	-	-	-	-	+	1	+	+	1	+	mild OJD	
32	-	-	-	-	-	-	c	-	-	-	-	-	-	-	-	uninfected/r	
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
35	+	-	+	-	?	-	-	1	+	-	-	-	-	1	+	mild OJD	
36	-	-	-	-	-	-	-	-	-	-	-	-	+	2(n)	+	mild OJD	
37	-	-	-	-	-	-	c	-	-	+	2	+	+	2(n)	+	mild OJD	
38	-	-	-	-	-	-	c	-	-	-	-	-	-	-	-	uninfected/r	
39	-	?	-	-	-	-	c	-	-	+	2(n)	+	+	?	+	mild OJD	
40	-	-	-	-	-	-	-	?	-	-	?	-	-	?	-	recovered	
41	-	-	-	-	-	-	c	-	-	-	-	-	+	-	+	mild OJD	
42	-	?	-	-	-	-	-	-	-	+	-	+	+	-	+	mild OJD	
43	-	?	-	-	-	-	-	-	-	-	?	-	+	2(n)	+	mild OJD	
44	-	-	-	-	-	-	c	-	-	-	-	-	+	?	+	mild OJD	
45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	
46	-	-	-	-	-	-	c	3a	+	+	3b	+	+	3b	+	died OJD	33m
47	-	-	-	-	-	-	-	-	-	-	1	+	-	-	+	mild OJD	
49	-	-	-	-	-	-	-	-	-	+	3b	+	+	3b/3c	+	severe OJD	
50	-	-	-	+	3c	+	c	3c	+	+	3c	+	+	3c	+	severe OJD	
51	-	-	-	-	1	+	c	-	-	-	-	-	-	-	-	recovered	
52	-	-	-	-	-	-	-	1	+	-	-	-	-	-	-	recovered	
53	-	?	-	-	-	-	+	3b	+	-	-	-	nd	-	-	dead, sever	25m
54	+	2	+	+	3c	+	+	3c	+	+		+	+	3a/c	+	died OJD	28m
55	-	-	-	-	-	-	-	-	-	-	-	-	+	3b	+	died OJD	24m
56	-	-	-	-	-	-	-	-	-	-	?	-	+	2(n)	+	mild OJD	
57	-	-	-	-	-	-	-	1	+	-	?	-	-	?	-	recovered	
58	+	-	+	-	-	-	+	3c	+	+	3b	+	+	3b	+	died OJD	34m
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	uninfected/r	

Appendix 3e. Summary of biopsy results: includes culture and/or histo positive

Sheep ID	Find Findings from biopsy												Necropsy		OJD status	Age at death	
	c	h	12	c	h	18	c	h	24	c	h	36*	c	h			
60	-	?	-				+	-	+	+	3a	+	+	3b/3c	+	severe OJD	
61	-	-	-				-	-	-	-	1	+	+	1	+	mild OJD	
62	-	-	-				-	-	-	-	-	-	+	-	+	mild OJD	
63	+	1	+	-	-	-	-	-	-	+	?	+	+	?	+	mild OJD	
64	-	-	-				+	-	+	-	-	-	-	-	-	recovered	
65	+	-	+	+	-	+	-	?	-	-	?	-	+	?	+	mild OJD	
66	-	-	-				-	-	-	-	-	-	+	-	+	mild OJD	
67	-	-	-				-	-	-	-	?	-	+	?	+	mild OJD	
68	-	-	-				-	-	-				nd			dead, uninfected	25m
69	-	-	-				-	-	-	-	-	-	-	-	-	uninfected/r	
70	+	-	+	-	?	-	-	-	-	+	?	+	+	-	+	mild OJD	
71	-	-	-				c	-	-	+	3a/3c	+	+	3b/3c	+	severe OJD	
72	+	-	+	-	?	-	-	-	-	-	?	-	-	?	-	recovered	
73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	recovered	
74	-	?	-				+	3b	+				+	3b	+	died OJD	28m
75	-	-	-				-	-	-	-	-	-	-	-	-	uninfected/r	
76	+	2	+	+	3c	+							+	3c	+	died OJD	24m
77	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	mild OJD	
78	+	-	+	+	2n	+	+	3b/3c	+	+	3b	+	+	3b	+	died OJD	29m
79	-	-	-				-	-	-	-	-	-	+	-	+	mild OJD	
80	-	-	-				-	-	-	+	2(n)	+	+	2	+	mild OJD	
81	-	-	-				-	-	-	-	-	-	-	?	-	uninfected/r	
90	-	-	-				-	-	-	-	-	-	-	-	-	uninfected/r	
Total +ve	15	4	15	8	8	9	12	17	19	19	20	23	33	22	36		
Total sheep	77	77	77	20	20	20	60	73	73	63	63	63	63	63	63		
% +ve	19	5.2	19.5	40	40.0	45.0	20	23.3	26.0	30.2	31.7	36.5	52.4	34.9	57.1		
For analysis:	15/74	4/74	15/74				11/58	16/71	18/71	19/63	20/63	23/63					

These sheep excluded from statistical analysis

Samples collected at early necropsy. Not included in % positive, total sheep, nor in statistical analysis

* These are mock biopsy samples collected during necropsy examination

** This sheep negative in the main necropsy histo, but positive in the mock biopsy

Appendix 4. Analyses of predictive values of tests

Appendix 4a. Analyses for ELISA.

Appendix 4b. Analyses for AGID

Appendix 4c. Analyses for IFN- γ

Appendix 4d. Analyses for skin testing

Appendix 4e. Analyses for IFC

Appendix 4f. Analyses for D-PCR

Appendix 4g. Analyses for serial IFC

Appendix 4h. Analyses for biopsy

Appendix 4i. Analyses for all routine tests in combination

Appendix 4a. Analyses for ELISA. In EpiInfo ver 6, Chi square test

All relative to final
infection status

18mths, 6 elisa+

ELISA	inf	uninf	tot	ELISA	dead	alive	tot	sens	
pos	5	1	6	pos	3	3	6	spec	0.96
neg	42	26	68	neg	8	60	68	ppv	0.83
tot	47	27	74	tot	11	63	74	npv	0.38
chisq:	1.11	P:	0.41*	chisq:	6.37	P:	0.04*	prev	0.64

24mths, 9 elisa+

ELISA	inf	uninf	tot	ELISA	dead	alive	tot	sens	
pos	6	3	9	pos	3	6	9	spec	0.89
neg	38	24	62	neg	5	57	62	ppv	0.67
tot	44	27	71	tot	8	63	71	npv	0.39
chisq:	0.1	P:	1.0*	chisq:	5.02	P:	0.06*	prev	0.62

30mths, 5 elisa+

ELISA	inf	uninf	tot	ELISA	dead	alive	tot	sens	
pos	5	0	5	pos	2	3	5	spec	1.00
neg	34	27	61	neg	1	60	61	ppv	1.00
tot	39	27	66	tot	3	63	66	npv	0.44
chisq:	3.75	P:	0.07*	chisq:	15.67	P:	0.01*	prev	0.59

36mths, 4 elisa +

ELISA	inf	uninf	tot	ELISA	dead	alive	tot	sens	
pos	4	0	4	pos				spec	1.00
neg	32	27	59	neg				ppv	1.00
tot	36	27	63	tot				npv	0.46
chisq:	3.2	P:	0.13*	chisq:				prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4b. Analyses for AGID. In EpiInfo ver 6, Chi square test

All relative to final infection status

9mths, 0agid+

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.00
pos	0	0	0	pos	0	0	0	spec	1.00
neg	47	27	74	neg	11	63	74	ppv	#DIV/0!
tot	47	27	74	tot	11	63	74	npv	0.36
chisq:		P:		chisq:		P:		prev	0.64

12mths, 0agid+

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.00
pos	0	0	0	pos	0	0	0	spec	1.00
neg	47	27	74	neg	11	63	74	ppv	#DIV/0!
tot	47	27	74	tot	11	63	74	npv	0.36
chisq:		P:		chisq:		P:		prev	0.64

18mths, 2agid+

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.02
pos	1	1	2	pos	1	1	2	spec	0.96
neg	46	26	72	neg	10	62	68	ppv	0.50
tot	47	27	74	tot	11	63	74	npv	0.36
chisq:	0.16	P:	1.0*	chisq:	2.01	P:	0.28*	prev	0.64

24mths, 2 agid+

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.05
pos	2	0	2	pos	2	0	2	spec	1.00
neg	42	27	69	neg	6	63	69	ppv	1.00
tot	44	27	71	tot	8	63	71	npv	0.39
chisq:	1.26	P:	0.52*	chisq:	16.21	P:	0.01*	prev	0.62

30mths, 4 agid +

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.08
pos	3	1	4	pos	2	2	4	spec	0.96
neg	36	26	62	neg	1	61	62	ppv	0.75
tot	39	27	66	tot	3	63	66	npv	0.42
chisq:	0.45	P:	0.64*	chisq:	20.28	P:	0.008*	prev	0.59

36mths, 2 agid +

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.06
pos	2	0	2	pos				spec	1.00
neg	34	27	61	neg				ppv	1.00
tot	36	27	63	tot				npv	0.44
chisq:	1.55	P:	0.5*	chisq:				prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4c. Analyses for Ifn-g. In EpiInfo ver 6, Chi square test

All relative to final infection status

9mths, 6ifn +

IFN	inf	uninf	tot	IFN	dead	alive	tot	sens	
pos	5	1	6	pos	2	4	6	spec	0.96
neg	42	26	68	neg	9	59	68	ppv	0.83
tot	47	27	74	tot	11	63	74	npv	0.38
chisq:	1.1	P:	0.41*	chisq:	1.76	P:	0.21*	prev	0.64

12mths, 1ifn +

IFN	inf	uninf	tot	IFN	dead	alive	tot	sens	
pos	1	0	1	pos	1	0	1	spec	1.00
neg	46	27	73	neg	10	63	73	ppv	1.00
tot	47	27	74	tot	11	63	74	npv	0.37
chisq:	0.58	P:	1.0*	chisq:	5.81	P:	.14*	prev	0.64

18mths, 9ifn +

IFN	inf	uninf	tot	IFN	dead	alive	tot	sens	
pos	8	1	9	pos	6	3	9	spec	0.96
neg	39	26	65	neg	5	60	65	ppv	0.89
tot	47	27	74	tot	11	63	74	npv	0.40
chisq:	2.85	P:	0.14*	chisq:	21.73	P:	.0001*	prev	0.64

24mths, 8ifn +

IFN	inf	uninf	tot	IFN	dead	alive	tot	sens	
pos	7	1	8	pos	4	4	8	spec	0.96
neg	37	26	63	neg	4	59	63	ppv	0.88
tot	44	27	71	tot	8	63	71	npv	0.41
chisq:	2.49	P:	0.14*	chisq:	13.53	P:	.004*	prev	0.62

30mths, 32ifn + (5 rec, 4 n inf, 14 mild, 6 sev, 3 died)

IFN	inf	uninf	tot	IFN	dead	alive	tot	sens	
pos	23	9	32	pos	3	29	32	spec	0.67
neg	16	18	34	neg	0	34	34	ppv	0.72
tot	39	27	66	tot	3	63	66	npv	0.53
chisq:	4.2	P:	0.04	chisq:	3.34	P:	0.11*	prev	0.59

36mths, 9ifn + (2rec, 1 n inf, 4 mild, 2 sev)

IFN	inf	uninf	tot	IFN	severe	other	tot	sens	
pos	6	3	9	pos	2	7	9	spec	0.89
neg	30	24	54	neg	4	50	54	ppv	0.67
tot	36	27	63	tot	6	57	63	npv	0.44
chisq:	0.39	P:	0.72*	chisq:	1.96	P:	0.20	prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4d. Analyses for skin testing. In EpiInfo ver 6, Chi square test

All relative to final infection status

24mths, 23 dth+ (5 rec, 2 n inf, 7 mild, 3 sev, 6 died)

DTH	inf	uninf	tot	DTH	dead	alive	tot	sens	
pos	16	7	23	pos	6	17	23	spec	0.74
neg	28	20	48	neg	2	46	48	ppv	0.70
tot	44	27	71	tot	8	63	71	npv	0.42
chisq:	83	P:	0.36	chisq:	7.47	P:	0.011*	prev	0.62

30mths, 8 dth+

DTH	inf	uninf	tot	DTH	dead	alive	tot	sens	
pos	6	2	8	pos	1	7	8	spec	0.93
neg	33	25	58	neg	2	56	58	ppv	0.75
tot	39	27	66	tot	3	63	66	npv	0.43
chisq:	0.95	P:	0.46*	chisq:	1.33	P:	0.33*	prev	0.59

36mths, 2 dth+

DTH	inf	uninf	tot	DTH	dead	alive	tot	sens	
pos	2	0	2	pos				spec	1.00
neg	34	27	61	neg				ppv	1.00
tot	36	27	63	tot				npv	0.44
chisq:	1.55	P:	0.5*	chisq:				prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4e. Analyses for IFC. In EpiInfo ver 6, Chi square test

All relative to final infection status

9mths, 8ifc + (4 rec, 1 mild, 1 sev, 2 died ojd)

IFC	inf	uninf	tot	IFC	dead	alive	tot	sens	
pos	4	4	8	pos	2	6	8	spec	0.85
neg	43	23	66	neg	9	57	66	ppv	0.50
tot	47	27	74	tot	11	63	74	npv	0.35
chisq:	0.71	P:	0.45*	chisq:	0.73	P:	0.39	prev	0.64

12mths, 1ifc + (rec)

IFC	inf	uninf	tot	IFC	dead	alive	tot	sens	
pos	0	1	1	pos	0	1	1	spec	0.96
neg	47	26	73	neg	11	62	73	ppv	0.00
tot	47	27	74	tot	11	63	74	npv	0.36
chisq:	1.73	P:	0.36*	chisq:	0.18	P:	0.85*	prev	0.64

18mths, 6ifc + (all died ojd)

IFC	inf	uninf	tot	IFC	dead	alive	tot	sens	
pos	6	0	6	pos	6	0	6	spec	1.00
neg	41	27	68	neg	5	63	68	ppv	1.00
tot	47	27	74	tot	11	63	74	npv	0.40
chisq:	3.75	P:	0.08*	chisq:	37.4	P:	0.0000*	prev	0.64

24mths, 7ifc + (all died ojd)

IFC	inf	uninf	tot	IFC	dead	alive	tot	sens	
pos	7	0	7	pos	7	0	7	spec	1.00
neg	37	27	64	neg	1	63	64	ppv	1.00
tot	44	27	71	tot	8	63	71	npv	0.42
chisq:	4.77	P:	0.04*	chisq:	61.5	P:	0.0000*	prev	0.62

30mths, 6ifc + (1 mild, 2 sev, 3 died)

IFC	inf	uninf	tot	IFC	dead	alive	tot	sens	
pos	6	0	6	pos	3	3	6	spec	1.00
neg	33	27	60	neg	0	60	60	ppv	1.00
tot	39	27	66	tot	3	63	66	npv	0.45
chisq:	4.57	P:	0.07*	chisq:	31.43	P:	0.0004*	prev	0.59

36mths, 9ifc + (4 mild, 5 sev)

IFC	inf	uninf	tot	IFC	severe	other	tot	sens	
pos	9	0	9	pos	5	4	9	spec	1.00
neg	25	27	54	neg	1	53	54	ppv	1.00
tot	36	27	63	tot	6	57	63	npv	0.50
chisq:	8.38	P:	0.003*	chisq:	25.82	P:	0.0001*	prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4f. Analyses for D-PCR. In EpiInfo ver 6, Chi square test

All relative to final infection status

9mths, 3dpcr + (2 rec, 1 died ojd)

dpcr	inf	uninf	tot	dpcr	dead	alive	tot	sens	0.02
pos	1	2	3	pos	1	2	3	spec	0.93
neg	46	25	71	neg	10	61	71	ppv	0.33
tot	47	27	74	tot	11	63	74	npv	0.35
chisq:	1.23	P:	0.30*	chisq:	0.84	P:	0.39*	prev	0.64

12mths, 3dpcr + (1rec, 2 mild)

dpcr	inf	uninf	tot	dpcr	dead	alive	tot	sens	0.04
pos	2	1	3	pos	0	3	3	spec	0.96
neg	45	26	71	neg	11	60	71	ppv	0.67
tot	47	27	74	tot	11	63	74	npv	0.37
chisq:	0.01	P:	0.7*	chisq:	0.55	P:	0.61*	prev	0.64

18mths, 2dpcr + (all died ojd)

dpcr	inf	uninf	tot	dpcr	dead	alive	tot	sens	0.04
pos	2	0	2	pos	2	0	2	spec	1.00
neg	45	27	72	neg	9	63	72	ppv	1.00
tot	47	27	74	tot	11	63	74	npv	0.38
chisq:	1.18	P:	0.4*	chisq:	11.77	P:	0.02*	prev	0.64

24mths, 6dpcr + (5died ojd, 1 severe)

dpcr	inf	uninf	tot	dpcr	dead	alive	tot	sens	0.14
pos	6	0	6	pos	5	1	6	spec	1.00
neg	38	27	65	neg	3	62	65	ppv	1.00
tot	44	27	71	tot	8	63	71	npv	0.42
chisq:	4.02	P:	0.049*	chisq:	34.04	P:	0.0000*	prev	0.62

30mths, 3dpcr + (1 mild, 2 died)

dpcr	inf	uninf	tot	dpcr	dead	alive	tot	sens	0.08
pos	3	0	3	pos	2	1	3	spec	1.00
neg	36	27	63	neg	1	62	63	ppv	1.00
tot	39	27	66	tot	3	63	66	npv	0.43
chisq:	2.18	P:	0.20*	chisq:	27.95	P:	0.004*	prev	0.59

36mths, 9ifc + (4 mild, 5 sev)

dpcr	inf	uninf	tot	dpcr	severe	other	tot	sens	0.25
pos	9	0	9	pos	5	4	9	spec	1.00
neg	25	27	54	neg	1	53	54	ppv	1.00
tot	36	27	63	tot	6	57	63	npv	0.50
chisq:	8.38	P:	0.003*	chisq:	25.82	P:	0.0001*	prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4g. Analyses for serial faecal culture. In EpiInfo ver 6, Chi square test

All relative to final infection status

9, 12, 18, 24, 30, 36 mths

	inf	uninf	tot		dead	alive	tot		
pos	22	5	27	pos	11	16	27	sens	0.47
neg	25	22	47	neg	0	47	47	spec	0.81
tot	47	27	74	tot	11	63	74	ppv	0.81
chisq:	5.92	P:	0.015	chisq:	22.5	P:	0.000	npv	0.47
								prev	0.64

18, 24, 30 mths

	inf	uninf	tot		dead	alive	tot		
pos	14	0	14	pos	11	3	14	sens	0.30
neg	33	27	60	neg	0	60	60	spec	1.00
tot	47	27	74	tot	11	63	74	ppv	1.00
chisq:	9.91	P:	0.002	chisq:	55.4	P:	0.000	npv	0.45
								prev	0.64

24, 30, 36

	inf	uninf	tot		dead	alive	tot		
pos	17	0	17	pos	8	9	17	sens	0.39
neg	27	27	54	neg	0	54	54	spec	1.00
tot	44	27	71	tot	8	63	71	ppv	1.00
chisq:	13.7	P:	0.000	chisq:	28.6	P:	0.000	npv	0.50
								prev	0.62

30, 36

	inf	uninf	tot		dead	alive	tot		
pos	12	0	12	pos	3	9	12	sens	0.31
neg	27	27	54	neg	0	54	54	spec	1.00
tot	39	27	66	tot	3	63	66	ppv	1.00
chisq:	10.2	P:	0.001	chisq:	14.14	P:	0.000*	npv	0.50
								prev	0.59

18, 24

	inf	uninf	tot		dead	alive	tot		
pos	10	0	10	pos				sens	0.21
neg	37	27	64	neg				spec	1.00
tot	47	27	74	tot				ppv	1.00
chisq:	6.6	P:	0.01	chisq:				npv	0.42
								prev	0.64

24, 30

	inf	uninf	tot		dead	alive	tot		
pos	11	0	11	pos				sens	0.25
neg	33	27	60	neg				spec	1.00
tot	44	27	71	tot				ppv	1.00
chisq:	10.2	P:	0.005	chisq:				npv	0.45
								prev	0.62

* used Fisher exact test (expected cell values <5)

Appendix 4h. Analyses for biopsy. In Epilnfo ver 6, Chi square test

All relative to final infection status

12mths, 15 biopsy + (4 rec, 6 mild, 5 died ojd)

BIOPSY	inf	uninf	tot	BIOPSY	dead	alive	tot	sens	
pos	11	4	15	pos	5	10	15	spec	0.85
neg	36	23	59	neg	6	53	59	ppv	0.73
tot	47	27	74	tot	11	63	74	npv	0.39
chisq:	0.78	P:	0.38	chisq:	5.07	P:	0.04*	prev	0.64

24mths, 19 biopsy + (4 rec, 3 mild, 3 sev, 8 died)

BIOPSY	inf	uninf	tot	BIOPSY	dead	alive	tot	sens	
pos	14	4	18	pos	8	10	18	spec	0.85
neg	30	23	53	neg	0	53	53	ppv	0.78
tot	44	27	71	tot	8	63	71	npv	0.43
chisq:	2.56	P:	0.11	chisq:	26.55	P:	0.0000*	prev	0.62

24mths (only sheep with uncontam cultures, 15 biopsy + (3 rec, 3 mild, 2 sev, 7 died)

BIOPSY	inf	uninf	tot	BIOPSY	dead	alive	tot	sens	
pos	12	3	15	pos	7	8	15	spec	0.86
neg	24	19	43	neg	0	43	43	ppv	0.80
tot	36	22	58	tot	7	51	58	npv	0.44
chisq:	2.76	P:	0.096	chisq:	22.82	P:	0.0000*	prev	0.62

36mths, 23 biopsy + (17 mild, 6 severe)

BIOPSY	inf	uninf	tot	BIOPSY	dead	alive	tot	sens	
pos	23	0	23	pos				spec	1.00
neg	13	27	40	neg				ppv	1.00
tot	36	27	63	tot				npv	0.68
chisq:	27.17	P:	0.0000	chisq:				prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4i. Analyses for all routine (IFC, D-PCR, AGID, ELISA, DTH or IFN-G. From minitab, Chi square test

All relative to final infection status

9mths, 12 pos (5 rec, 3 died ojd, 1 severe, 3 mild)

	inf	uninf	tot		dead	alive	tot		
pos	7	5	12	pos	3	9	12	sens	0.15
neg	40	22	62	neg	8	54	62	spec	0.81
tot	47	27	74	tot	11	63	74	ppv	0.58
chisq:	0.166	P:	0.68	chisq:	1.16	P:	0.28	npv	0.35
								prev	0.64

12mths, 5 + (1died, 2 mild, 2 rec)

	inf	uninf	tot		dead	alive	tot		
pos	3	2	5	pos	1	4	5	sens	0.06
neg	44	25	69	neg	10	59	69	spec	0.93
tot	47	27	74	tot	11	63	74	ppv	0.60
chisq:	0.02	P:	0.86	chisq:	0.112	P:	0.56*	npv	0.36
								prev	0.64

18mths, 13 + (8 died ojd, 3 mild, 1 severe, 1 rec)

	inf	uninf	tot		dead	alive	tot		
pos	12	1	13	pos	8	5	13	sens	0.26
neg	35	26	71	neg	3	58	71	spec	0.96
tot	47	27	74	tot	11	63	74	ppv	0.92
chisq:	5.64	P:	0.018	chisq:	27.1	P:	0.000	npv	0.37
								prev	0.64

24mths, 26 + (8died ojd, 3 severe, 5 rec, 8 mild, 2 uninf)

	inf	uninf	tot		dead	alive	tot		
pos	19	7	26	pos	8	18	26	sens	0.43
neg	25	20	45	neg	0	45	65	spec	0.74
tot	44	27	71	tot	8	63	71	ppv	0.73
chisq:	2.14	P:	0.143	chisq:	15.6	P:	0.000	npv	0.44
								prev	0.62

30mths, 36 + (3 died, 17 mild, 6 severe, 6 rec, 4 uninf)

	inf	uninf	tot		dead	alive	tot		
pos	26	10	36	pos	3	33	36	sens	0.67
neg	13	17	30	neg	0	30	63	spec	0.63
tot	39	27	66	tot	3	63	66	ppv	0.72
chisq:	5.6	P:	0.017	chisq:	2.6	P:	0.106	npv	0.57
								prev	0.59

36mths, 16 + (7 mild, 6 sev, 2 rec, 1 uninf)

	inf	uninf	tot		severe	other	tot		
pos	13	3	16	pos				sens	0.36
neg	23	24	47	neg				spec	0.89
tot	36	27	63	tot				ppv	0.81
chisq:	5.09	P:	0.024	chisq:				npv	0.51
								prev	0.57

* used Fisher exact test (expected cell values <1)

Appendix 4b. Analyses for AGID. In EpiInfo ver 6, Chi square test

All relative to final infection status

9mths, 0agid+

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.00
pos	0	0	0	pos	0	0	0	spec	1.00
neg	47	27	74	neg	11	63	74	ppv	#DIV/0!
tot	47	27	74	tot	11	63	74	npv	0.36
chisq:		P:		chisq:		P:		prev	0.64

12mths, 0agid+

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.00
pos	0	0	0	pos	0	0	0	spec	1.00
neg	47	27	74	neg	11	63	74	ppv	#DIV/0!
tot	47	27	74	tot	11	63	74	npv	0.36
chisq:		P:		chisq:		P:		prev	0.64

18mths, 2agid+

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.02
pos	1	1	2	pos	1	1	2	spec	0.96
neg	46	26	72	neg	10	62	68	ppv	0.50
tot	47	27	74	tot	11	63	74	npv	0.36
chisq:	0.16	P:	1.0*	chisq:	2.01	P:	0.28*	prev	0.64

24mths, 2 agid+

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.05
pos	2	0	2	pos	2	0	2	spec	1.00
neg	42	27	69	neg	6	63	69	ppv	1.00
tot	44	27	71	tot	8	63	71	npv	0.39
chisq:	1.26	P:	0.52*	chisq:	16.21	P:	0.01*	prev	0.62

30mths, 4 agid +

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.08
pos	3	1	4	pos	2	2	4	spec	0.96
neg	36	26	62	neg	1	61	62	ppv	0.75
tot	39	27	66	tot	3	63	66	npv	0.42
chisq:	0.45	P:	0.64*	chisq:	20.28	P:	0.008*	prev	0.59

36mths, 2 agid +

AGID	inf	uninf	tot	AGID	dead	alive	tot	sens	0.06
pos	2	0	2	pos				spec	1.00
neg	34	27	61	neg				ppv	1.00
tot	36	27	63	tot				npv	0.44
chisq:	1.55	P:	0.5*	chisq:				prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4c. Analyses for Ifn-g. In EpiInfo ver 6, Chi square test

All relative to final infection status

9mths, 6ifn +

IFN	inf	uninf	tot	IFN	dead	alive	tot	sens	
pos	5	1	6	pos	2	4	6	spec	0.96
neg	42	26	68	neg	9	59	68	ppv	0.83
tot	47	27	74	tot	11	63	74	npv	0.38
chisq:	1.1	P:	0.41*	chisq:	1.76	P:	0.21*	prev	0.64

12mths, 1ifn +

IFN	inf	uninf	tot	IFN	dead	alive	tot	sens	
pos	1	0	1	pos	1	0	1	spec	1.00
neg	46	27	73	neg	10	63	73	ppv	1.00
tot	47	27	74	tot	11	63	74	npv	0.37
chisq:	0.58	P:	1.0*	chisq:	5.81	P:	.14*	prev	0.64

18mths, 9ifn +

IFN	inf	uninf	tot	IFN	dead	alive	tot	sens	
pos	8	1	9	pos	6	3	9	spec	0.96
neg	39	26	65	neg	5	60	65	ppv	0.89
tot	47	27	74	tot	11	63	74	npv	0.40
chisq:	2.85	P:	0.14*	chisq:	21.73	P:	.0001*	prev	0.64

24mths, 8ifn +

IFN	inf	uninf	tot	IFN	dead	alive	tot	sens	
pos	7	1	8	pos	4	4	8	spec	0.96
neg	37	26	63	neg	4	59	63	ppv	0.88
tot	44	27	71	tot	8	63	71	npv	0.41
chisq:	2.49	P:	0.14*	chisq:	13.53	P:	.004*	prev	0.62

30mths, 32ifn + (5 rec, 4 n inf, 14 mild, 6 sev, 3 died)

IFN	inf	uninf	tot	IFN	dead	alive	tot	sens	
pos	23	9	32	pos	3	29	32	spec	0.67
neg	16	18	34	neg	0	34	34	ppv	0.72
tot	39	27	66	tot	3	63	66	npv	0.53
chisq:	4.2	P:	0.04	chisq:	3.34	P:	0.11*	prev	0.59

36mths, 9ifn + (2rec, 1 n inf, 4 mild, 2 sev)

IFN	inf	uninf	tot	IFN	severe	other	tot	sens	
pos	6	3	9	pos	2	7	9	spec	0.89
neg	30	24	54	neg	4	50	54	ppv	0.67
tot	36	27	63	tot	6	57	63	npv	0.44
chisq:	0.39	P:	0.72*	chisq:	1.96	P:	0.20	prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4d. Analyses for skin testing. In EpiInfo ver 6, Chi square test

All relative to final infection status

24mths, 23 dth+ (5 rec, 2 n inf, 7 mild, 3 sev, 6 died)

DTH	inf	uninf	tot	DTH	dead	alive	tot	sens	
pos	16	7	23	pos	6	17	23	spec	0.74
neg	28	20	48	neg	2	46	48	ppv	0.70
tot	44	27	71	tot	8	63	71	npv	0.42
chisq:	83	P:	0.36	chisq:	7.47	P:	0.011*	prev	0.62

30mths, 8 dth+

DTH	inf	uninf	tot	DTH	dead	alive	tot	sens	
pos	6	2	8	pos	1	7	8	spec	0.93
neg	33	25	58	neg	2	56	58	ppv	0.75
tot	39	27	66	tot	3	63	66	npv	0.43
chisq:	0.95	P:	0.46*	chisq:	1.33	P:	0.33*	prev	0.59

36mths, 2 dth+

DTH	inf	uninf	tot	DTH	dead	alive	tot	sens	
pos	2	0	2	pos				spec	1.00
neg	34	27	61	neg				ppv	1.00
tot	36	27	63	tot				npv	0.44
chisq:	1.55	P:	0.5*	chisq:				prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4e. Analyses for IFC. In EpiInfo ver 6, Chi square test

All relative to final infection status

9mths, 8ifc + (4 rec, 1 mild, 1 sev, 2 died ojd)

IFC	inf	uninf	tot	IFC	dead	alive	tot	sens	
pos	4	4	8	pos	2	6	8	spec	0.85
neg	43	23	66	neg	9	57	66	ppv	0.50
tot	47	27	74	tot	11	63	74	npv	0.35
chisq:	0.71	P:	0.45*	chisq:	0.73	P:	0.39	prev	0.64

12mths, 1ifc + (rec)

IFC	inf	uninf	tot	IFC	dead	alive	tot	sens	
pos	0	1	1	pos	0	1	1	spec	0.96
neg	47	26	73	neg	11	62	73	ppv	0.00
tot	47	27	74	tot	11	63	74	npv	0.36
chisq:	1.73	P:	0.36*	chisq:	0.18	P:	0.85*	prev	0.64

18mths, 6ifc + (all died ojd)

IFC	inf	uninf	tot	IFC	dead	alive	tot	sens	
pos	6	0	6	pos	6	0	6	spec	1.00
neg	41	27	68	neg	5	63	68	ppv	1.00
tot	47	27	74	tot	11	63	74	npv	0.40
chisq:	3.75	P:	0.08*	chisq:	37.4	P:	0.0000*	prev	0.64

24mths, 7ifc + (all died ojd)

IFC	inf	uninf	tot	IFC	dead	alive	tot	sens	
pos	7	0	7	pos	7	0	7	spec	1.00
neg	37	27	64	neg	1	63	64	ppv	1.00
tot	44	27	71	tot	8	63	71	npv	0.42
chisq:	4.77	P:	0.04*	chisq:	61.5	P:	0.0000*	prev	0.62

30mths, 6ifc + (1 mild, 2 sev, 3 died)

IFC	inf	uninf	tot	IFC	dead	alive	tot	sens	
pos	6	0	6	pos	3	3	6	spec	1.00
neg	33	27	60	neg	0	60	60	ppv	1.00
tot	39	27	66	tot	3	63	66	npv	0.45
chisq:	4.57	P:	0.07*	chisq:	31.43	P:	0.0004*	prev	0.59

36mths, 9ifc + (4 mild, 5 sev)

IFC	inf	uninf	tot	IFC	severe	other	tot	sens	
pos	9	0	9	pos	5	4	9	spec	1.00
neg	25	27	54	neg	1	53	54	ppv	1.00
tot	36	27	63	tot	6	57	63	npv	0.50
chisq:	8.38	P:	0.003*	chisq:	25.82	P:	0.0001*	prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4f. Analyses for D-PCR. In EpiInfo ver 6, Chi square test

All relative to final infection status

9mths, 3dpcr + (2 rec, 1 died ojd)

dpcr	inf	uninf	tot	dpcr	dead	alive	tot	sens	0.02
pos	1	2	3	pos	1	2	3	spec	0.93
neg	46	25	71	neg	10	61	71	ppv	0.33
tot	47	27	74	tot	11	63	74	npv	0.35
chisq:	1.23	P:	0.30*	chisq:	0.84	P:	0.39*	prev	0.64

12mths, 3dpcr + (1rec, 2 mild)

dpcr	inf	uninf	tot	dpcr	dead	alive	tot	sens	0.04
pos	2	1	3	pos	0	3	3	spec	0.96
neg	45	26	71	neg	11	60	71	ppv	0.67
tot	47	27	74	tot	11	63	74	npv	0.37
chisq:	0.01	P:	0.7*	chisq:	0.55	P:	0.61*	prev	0.64

18mths, 2dpcr + (all died ojd)

dpcr	inf	uninf	tot	dpcr	dead	alive	tot	sens	0.04
pos	2	0	2	pos	2	0	2	spec	1.00
neg	45	27	72	neg	9	63	72	ppv	1.00
tot	47	27	74	tot	11	63	74	npv	0.38
chisq:	1.18	P:	0.4*	chisq:	11.77	P:	0.02*	prev	0.64

24mths, 6dpcr + (5died ojd, 1 severe)

dpcr	inf	uninf	tot	dpcr	dead	alive	tot	sens	0.14
pos	6	0	6	pos	5	1	6	spec	1.00
neg	38	27	65	neg	3	62	65	ppv	1.00
tot	44	27	71	tot	8	63	71	npv	0.42
chisq:	4.02	P:	0.049*	chisq:	34.04	P:	0.0000*	prev	0.62

30mths, 3dpcr + (1 mild, 2 died)

dpcr	inf	uninf	tot	dpcr	dead	alive	tot	sens	0.08
pos	3	0	3	pos	2	1	3	spec	1.00
neg	36	27	63	neg	1	62	63	ppv	1.00
tot	39	27	66	tot	3	63	66	npv	0.43
chisq:	2.18	P:	0.20*	chisq:	27.95	P:	0.004*	prev	0.59

36mths, 9ifc + (4 mild, 5 sev)

dpcr	inf	uninf	tot	dpcr	severe	other	tot	sens	0.25
pos	9	0	9	pos	5	4	9	spec	1.00
neg	25	27	54	neg	1	53	54	ppv	1.00
tot	36	27	63	tot	6	57	63	npv	0.50
chisq:	8.38	P:	0.003*	chisq:	25.82	P:	0.0001*	prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4g. Analyses for serial faecal culture. In EpiInfo ver 6, Chi square test

All relative to final infection status

9, 12, 18, 24, 30, 36 mths

	inf	uninf	tot		dead	alive	tot		
pos	22	5	27	pos	11	16	27	sens	0.47
neg	25	22	47	neg	0	47	47	spec	0.81
tot	47	27	74	tot	11	63	74	ppv	0.81
chisq:	5.92	P:	0.015	chisq:	22.5	P:	0.000	npv	0.47
								prev	0.64

18, 24, 30 mths

	inf	uninf	tot		dead	alive	tot		
pos	14	0	14	pos	11	3	14	sens	0.30
neg	33	27	60	neg	0	60	60	spec	1.00
tot	47	27	74	tot	11	63	74	ppv	1.00
chisq:	9.91	P:	0.002	chisq:	55.4	P:	0.000	npv	0.45
								prev	0.64

24, 30, 36

	inf	uninf	tot		dead	alive	tot		
pos	17	0	17	pos	8	9	17	sens	0.39
neg	27	27	54	neg	0	54	54	spec	1.00
tot	44	27	71	tot	8	63	71	ppv	1.00
chisq:	13.7	P:	0.000	chisq:	28.6	P:	0.000	npv	0.50
								prev	0.62

30, 36

	inf	uninf	tot		dead	alive	tot		
pos	12	0	12	pos	3	9	12	sens	0.31
neg	27	27	54	neg	0	54	54	spec	1.00
tot	39	27	66	tot	3	63	66	ppv	1.00
chisq:	10.2	P:	0.001	chisq:	14.14	P:	0.000*	npv	0.50
								prev	0.59

18, 24

	inf	uninf	tot		dead	alive	tot		
pos	10	0	10	pos				sens	0.21
neg	37	27	64	neg				spec	1.00
tot	47	27	74	tot				ppv	1.00
chisq:	6.6	P:	0.01	chisq:				npv	0.42
								prev	0.64

24, 30

	inf	uninf	tot		dead	alive	tot		
pos	11	0	11	pos				sens	0.25
neg	33	27	60	neg				spec	1.00
tot	44	27	71	tot				ppv	1.00
chisq:	10.2	P:	0.005	chisq:				npv	0.45
								prev	0.62

* used Fisher exact test (expected cell values <5)

Appendix 4h. Analyses for biopsy. In Epilnfo ver 6, Chi square test

All relative to final infection status

12mths, 15 biopsy + (4 rec, 6 mild, 5 died ojd)

BIOPSY	inf	uninf	tot	BIOPSY	dead	alive	tot	sens	
pos	11	4	15	pos	5	10	15	spec	0.85
neg	36	23	59	neg	6	53	59	ppv	0.73
tot	47	27	74	tot	11	63	74	npv	0.39
chisq:	0.78	P:	0.38	chisq:	5.07	P:	0.04*	prev	0.64

24mths, 19 biopsy + (4 rec, 3 mild, 3 sev, 8 died)

BIOPSY	inf	uninf	tot	BIOPSY	dead	alive	tot	sens	
pos	14	4	18	pos	8	10	18	spec	0.85
neg	30	23	53	neg	0	53	53	ppv	0.78
tot	44	27	71	tot	8	63	71	npv	0.43
chisq:	2.56	P:	0.11	chisq:	26.55	P:	0.0000*	prev	0.62

24mths (only sheep with uncontam cultures, 15 biopsy + (3 rec, 3 mild, 2 sev, 7 died)

BIOPSY	inf	uninf	tot	BIOPSY	dead	alive	tot	sens	
pos	12	3	15	pos	7	8	15	spec	0.86
neg	24	19	43	neg	0	43	43	ppv	0.80
tot	36	22	58	tot	7	51	58	npv	0.44
chisq:	2.76	P:	0.096	chisq:	22.82	P:	0.0000*	prev	0.62

36mths, 23 biopsy + (17 mild, 6 severe)

BIOPSY	inf	uninf	tot	BIOPSY	dead	alive	tot	sens	
pos	23	0	23	pos				spec	1.00
neg	13	27	40	neg				ppv	1.00
tot	36	27	63	tot				npv	0.68
chisq:	27.17	P:	0.0000	chisq:				prev	0.57

* used Fisher exact test (expected cell values <5)

Appendix 4i. Analyses for all routine (IFC, D-PCR, AGID, ELISA, DTH or IFN-G. From minitab, Chi square test

All relative to final infection status

9mths, 12 pos (5 rec, 3 died ojd, 1 severe, 3 mild)

	inf	uninf	tot		dead	alive	tot		
pos	7	5	12	pos	3	9	12	sens	0.15
neg	40	22	62	neg	8	54	62	spec	0.81
tot	47	27	74	tot	11	63	74	ppv	0.58
chisq:	0.166	P:	0.68	chisq:	1.16	P:	0.28	npv	0.35
								prev	0.64

12mths, 5 + (1died, 2 mild, 2 rec)

	inf	uninf	tot		dead	alive	tot		
pos	3	2	5	pos	1	4	5	sens	0.06
neg	44	25	69	neg	10	59	69	spec	0.93
tot	47	27	74	tot	11	63	74	ppv	0.60
chisq:	0.02	P:	0.86	chisq:	0.112	P:	0.56*	npv	0.36
								prev	0.64

18mths, 13 + (8 died ojd, 3 mild, 1 severe, 1 rec)

	inf	uninf	tot		dead	alive	tot		
pos	12	1	13	pos	8	5	13	sens	0.26
neg	35	26	71	neg	3	58	71	spec	0.96
tot	47	27	74	tot	11	63	74	ppv	0.92
chisq:	5.64	P:	0.018	chisq:	27.1	P:	0.000	npv	0.37
								prev	0.64

24mths, 26 + (8died ojd, 3 severe, 5 rec, 8 mild, 2 uninf)

	inf	uninf	tot		dead	alive	tot		
pos	19	7	26	pos	8	18	26	sens	0.43
neg	25	20	45	neg	0	45	65	spec	0.74
tot	44	27	71	tot	8	63	71	ppv	0.73
chisq:	2.14	P:	0.143	chisq:	15.6	P:	0.000	npv	0.44
								prev	0.62

30mths, 36 + (3 died, 17 mild, 6 severe, 6 rec, 4 uninf)

	inf	uninf	tot		dead	alive	tot		
pos	26	10	36	pos	3	33	36	sens	0.67
neg	13	17	30	neg	0	30	63	spec	0.63
tot	39	27	66	tot	3	63	66	ppv	0.72
chisq:	5.6	P:	0.017	chisq:	2.6	P:	0.106	npv	0.57
								prev	0.59

36mths, 16 + (7 mild, 6 sev, 2 rec, 1 uninf)

	inf	uninf	tot		severe	other	tot		
pos	13	3	16	pos				sens	0.36
neg	23	24	47	neg				spec	0.89
tot	36	27	63	tot				ppv	0.81
chisq:	5.09	P:	0.024	chisq:				npv	0.51
								prev	0.57

* used Fisher exact test (expected cell values <1)