Relationships between clinical signs, pathological changes and tissue distribution of *Mycobacterium avium* subspecies *paratuberculosis* in 21 cows from herds affected by Johne's disease

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Twenty-one cows from eight herds affected by Johne's disease were assigned to four groups: seven were not thriving and had persistent diarrhoea, six were not thriving and had intermittent diarrhoea, four were not thriving but did not have diarrhoea, and four were clinically normal. Postmortem, macroscopic lesions consistent with Johne's disease were identified in 17 of the cows and *Mycobacterium avium* subspecies *paratuberculosis* (MAP) was isolated from all of them. However, except for the fact that diarrhoea was correlated with the presence of lesions in the large intestine there was little correlation between the presence or absence of clinical signs and the lesions associated with Johne's disease. The tissue distribution of MAP was also poorly correlated with either the clinical signs or the lesions. The organism was widely distributed in 17 of the 21 cows, including three of the clinically normal animals, and was present in the mammary tissues of seven cows including two of the clinically normal animals. Three distinct histopathological patterns were observed in the affected intestines: infiltration of the lamina propria with giant cells, tuberculoid lesions, and lepromatous lesions; the lepromatous lesions were associated with extensive pathological changes.

JOHNE'S disease is a granulomatous enteritis of adult cattle, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Affected cattle usually remain bright and alert, but they typically fail to thrive and often develop intermittent or persistent diarrhoea. Chiodini and others (1984a) reported that the relationship between the extent of any lesions and the clinical signs was poor, but there has been little attempt to investigate the relationship in detail. Beurgelt and others (1978) described the gross and histopathological lesions present postmortem in 51 cows infected with MAP but they did not fully relate the nature of the animals' clinical signs to the nature and extent of their lesions.

The aim of this investigation was to examine the relationships between the clinical signs, the extent and character of the pathological changes and the tissue distribution of MAP in infected cattle.

MATERIALS AND METHODS

Twenty-one cows were acquired from eight herds in which Johne's disease had been confirmed or suspected. Seventeen of the cows came from four herds in which clinical Johne's disease had been a problem for several years, and the other four came from four different affected herds. The cows were transported to the Central Veterinary Research Laboratory (CVRL), Ireland, where they were held for one month before being slaughtered. While at the CVRL, their appetites, demeanour and faecal consistency were recorded three times a week and they were assigned to four groups (A, B, C and D) on the basis of their clinical history during the month; five of the animals had to be slaughtered during the month, and their clinical history on the farms of origin was also taken into account in making the assessments. The seven cows with illthrift and persistent diarrhoea were assigned to group A, the six with illthrift and intermittent diarrhoea to group B, the four with illthrift but no diarrhoea to group C and the four clinically normal cows were assigned to group D.

Serum obtained from 18 of the 21 animals while they were at the CVRL was analysed by an indirect ELISA (Parachek Johne's absorbed ELISA; Commonwealth Serum Laboratories) as described by O'Doherty and others (2002).

On the day of slaughter, each animal was examined and scored for body condition on a five-point scale (Wildman and others 1982). The clinical indicators assessed included demeanour, appetite, heart rate, respiration rate and temperature. Serum samples obtained on the day of slaughter were analysed with a photometric random-access clinical analyser (Cobas Mira Plus; Roche) in association with Randox kits to measure serum albumin and serum total protein concentrations; serum globulin was measured as the difference between the serum albumin and total protein levels.

Postmortem examination

The cows were euthanased with 40 per cent sodium pentabarbitone infused rapidly intravenously to anaesthetise them before they were exsanguinated and placed lying on their left side. The skin, abdominal muscles and peritoneum over the right abdominal wall were then reflected to gain access to the abdominal cavity. The intestines were examined and palpated immediately and, using aseptic techniques, portions of tissue were removed as quickly as possible from the mesenteric lymph nodes, the duodenum, the proximal, mid and terminal jejunum, the terminal ileum, the caecum and the proximal, spiral and terminal colon. The pieces of tissue were examined grossly and each piece was divided into two, one piece being fixed in formalin for processing for histopathology, the other being retained in a sterile container for bacteriological examination.

The rest of the carcase was then examined. The organs and associated lymph nodes from the thorax and abdomen, the reproductive organs and associated lymph nodes, and the lymph nodes from the head and limbs were removed, examined grossly and processed for bacteriological and histopathological examinations. The gloves and sterile scalpels used were changed between the handling of each sample.

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TABLE 1: Clinical signs, blood protein concentrations and ELISA results for Johne's disease in 21 cows from eight herds affected by the disease

Animal*	Appetite	Demeanour	Condition score†	Globulin (g/l) (reference 31-51)	Protein (g/l) (reference 57-83)	Albumin (g/l) (reference 23-37)	ELISA (OD)	ELISA result
A1	Poor	Dull	0-1	46·4	63·4	17·0	NA	NA
A2	Normal	Bright	0-1	33·8	50·3	16·5	2·559	+
A3	Poor	Dull	0-1	44·8	68·2	23·4	0·694	+
A4	Poor	Dull	0-1	32·9	61·7	28·7	NA	NA
A5	Poor	Dull	0-1	NA	NA	NA	0·659	+
A6	Normal	Bright	0-1	50·8	74·2	23·4	0·571	+
A7	Normal	Bright	1-2	34·8	47·9	13·1	NA	NA
B1	Poor	Dull	2-3	39·4	51·1	11·7	0·392	+
B2	Normal	Bright	1-2	56·1	84·4	28·3	0·355	+
B3	Normal	Bright	1-2	41·3	61·7	20·4	0·812	+
B4	Normal	Bright	1-2	49·0	73·3	24·0	0·765	+
B5	Normal	Bright	1-2	41·7	55·7	14·0	0·667	+
B6	Normal	Bright	1-2	49·8	64·0	14·2	0·791	+
C1	Poor	Dull	1-2	34·5	47·5	13·0	0·556	+
C2	Poor	Dull	1-2	NA	NA	NA	0·133	–
C3	Poor	Dull	1-2	79·1	99·9	20·8	0·142	–
C4	Normal	Bright	1-2	41·4	66·5	25·1	0·646	+
D1	Normal	Bright	2-3	52·6	80·6	28·0	0·989	+
D2	Normal	Bright	2-3	39·0	68·6	29·5	1·191	+
D3	Normal	Bright	2-3	NA	NA	NA	0·776	+
D4	Normal	Bright	2-3	NA	NA	NA	1·947	+

* Group A Persistent diarrhoea, Group B Intermittent diarrhoea, Group C No diarrhoea but illthriven, Group D Clinically normal
† Wildman and others 1982
NA Not available

For the bacteriological examination portions of each tissue were grouped into 11 pools as follows: lymph tissues from the head (tonsils, submandibular, parotid and retropharyngeal lymph nodes); thoracic contents (lungs, heart, bronchial and mediastinal lymph nodes); caecal contents; terminal ileum; mesenteric lymph nodes; ileocaecal lymph nodes; small intestine (duodenum, proximal, mid and terminal jejunum); large intestine (caecum proximal, mid and terminal colon); abdomen (spleen, hepatic lymph node, kidneys and liver); prescapular and popliteal lymph nodes; supramammary lymph nodes and mammary glands.

Portions of intestine were placed in a sterile petri dish and incised to expose the luminal surface which was then scraped lightly with a sterile scalpel to expose the mucosa; 3 to 5 g of material was then scraped from the mucosal surface and transferred to a sterile universal container. Lymph nodes and organs were placed in a sterile petri dish and cut into small pieces with a sterile scalpel.

Each sample for culture was transferred into a stomacher bag, 20 ml sterile phosphate-buffered saline was added and the material was homogenised in a 400 stomacher (Seward) at the high setting for four minutes. The homogenate was transferred to a sterile universal container and centrifuged at 3000 *g* for 25 minutes. After discarding the supernatant, the deposits were decontaminated with hexadecylpyridinium chloride, cultured in BACTEC medium and typed as described by O'Doherty and others (2002).

The distribution of MAP tissues was categorised as localised, intermediate or widespread; localised indicated that the organism was isolated only from the intestine and associated lymph nodes, intermediate that it was isolated from other tissues within the abdominal cavity, and widespread that it was isolated from tissues beyond the abdominal cavity.

Histopathology

The formalin-fixed portions of each of the tissues were embedded in paraffin and sectioned with a microtome at 4 µm. All the sections were stained with haematoxylin and eosin, and selected sections were stained with the Ziehl-Nielsen stain.

RESULTS

Group A

The clinical signs, blood protein levels and Johne's ELISA results for the seven animals in group A are summarised in Table 1.

In all of them, the lymphatics of the mesenteric serosa were dilated (Fig 1a) and the mesenteric lymph nodes were enlarged. In addition, they all had gross and histopathological changes typical of Johne's disease in both the large and small intestines. The gross changes were characterised by thickening and corrugation of the intestinal mucosa (Fig 1b) and the histopathological changes took three forms: a diffuse infiltration of macrophages into the mucosa and submucosa (lepromatous infiltration) (Fig 2), multifocal granulomatous infiltration of the intestinal mucosa accompanied by a diffuse infiltration of lymphocytes (tuberculoid infiltration) (Fig 3), and an infiltration of the intestinal lamina propria with Langhans' giant cells (Fig 4). The thickening and corrugation of the intestinal mucosa extended from the proximal jejunum to the mid-colon in cows A4, A5, A6 and A7, and from the proximal jejunum to the terminal colon in A1, A2 and A3. Tuberculoid lesions only were detectable in A4 and A6, tuberculoid and scattered giant cells were both detectable in A1 and A7, there were tuberculoid and lepromatous lesions in A2 and A3, and all three types of lesion were present in A5. The pathological changes are summarised in Table 2.

MAP was distributed widely throughout the tissues of all seven animals (Table 3).

Group B

The clinical signs, blood protein levels and Johne's ELISA results for the six animals in group B are summarised in Table 1.

The intestinal mucosa of cows B1, B3, B4, B5 and B6 was thickened and corrugated, but no lesions were visible in B2. The mesenteric lymph nodes of B1, B3, B4, B5 and B6 were enlarged and the serosal lymphatics were visibly dilated in B3, B4, B5 and B6. Macroscopic lesions were confined to the small intestine in B1 and B5 but present in both the small and

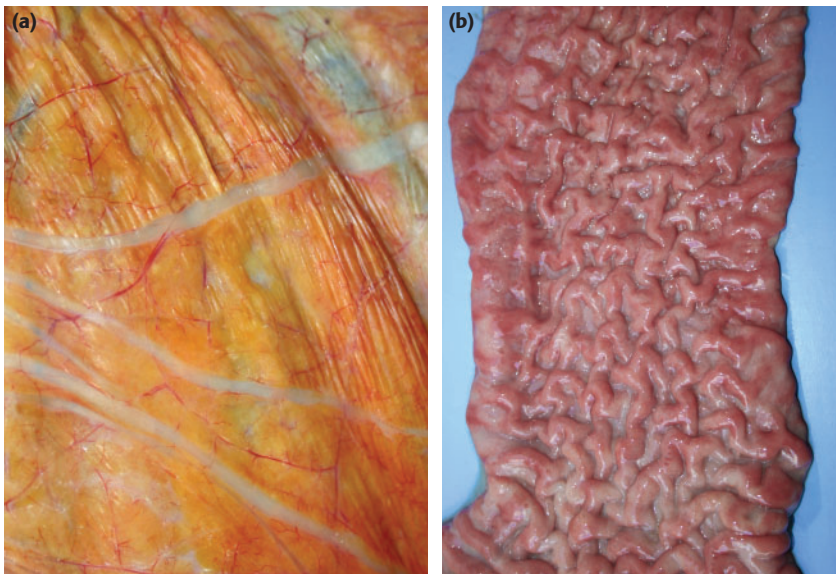

FIG 1: (a) Dilation of the lymphatics of the mesenteric serosa and (b) thickening and corrugation of the intestinal mucosa in cows with Johne's disease

large intestine of B3, B4 and B6. In B1 the lesions extended from the proximal jejunum to the terminal ileum, and in B5 from the mid-jejunum to the terminal ileum. In B4 the lesions extended from the proximal jejunum to the terminal colon, in B3 from the terminal jejunum to the terminal colon, and in B6 from the proximal jejunum to the caecum. There were scattered giant cells within the intestinal mucosa of B1, lepromatous lesions only were detected in B4 and B6 and both scattered giant cells and tuberculoid lesions were detected in B3 and B5. The pathological changes are summarised in Table 2.

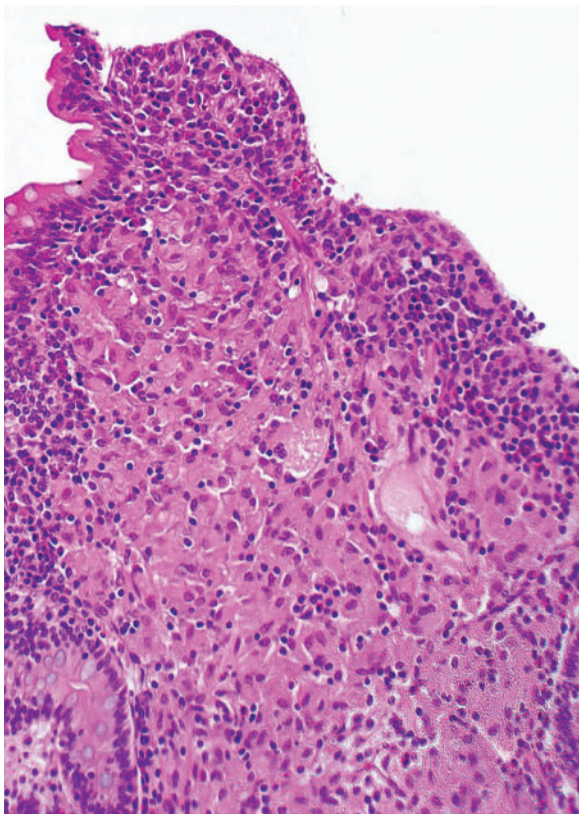

FIG 2: Lepromatous granulomatous inflammation in the terminal ileum of a cow with Johne's disease. Haematoxylin and eosin. x **400**

MAP was distributed widely throughout the tissues of B1, B2, B3, B4 and B5, but its distribution was localised in B6 (Table 3).

Group C

The clinical signs, blood protein levels and Johne's ELISA results for the four cows in group C are summarised in Table 1.

The intestinal mucosa of C1 and C4 was thickened and corrugated; C1 had enlarged lymph nodes and dilated serosal lymphatics and the macroscopic lesions extended from the proximal jejunum to the proximal colon. In C4, the lesions were confined to the small intestine where they extended from the mid-jejunum to the terminal ileum. In both these cows, only tuberculoid lesions were detected histologically. No lesions suggestive of Johne's disease were detected in C2 and C3; C2 had chronic mastitis and C3 had valvular endocarditis. The pathological changes are summarised in Table 2.

MAP was distributed widely throughout the tissues of C1 and C2, its distribution was localised in C4, and it was isolated only from the tissue pool derived from the prescapular and popliteal lymph nodes in C3 (the cow with endocarditis) (Table 3).

Group D

The clinical signs, blood protein levels and Johne's ELISA results for the four cows in group D are summarised in Table 1.

The intestinal mucosa of D2, D3 and D4 was thickened and corrugated but no significant lesions of any kind were identified in D1. The lymph nodes of the three affected animals were enlarged, but there was no dilation or milkiness of the serosal lymphatics. In D2 the lesions extended from the mid-jejunum to the terminal ileum, and in D3 and D4 from the terminal jejunum to the terminal ileum. Only scattered mucosal giant cells were detected in D2 and D3, and only tuberculoid lesions were detected in D4. The pathological changes are summarised in Table 2.

MAP was distributed widely throughout the tissues of D1, the cow that had no significant lesions, and D2 and D4. Its distribution was localised in D3 (Table 3).

DISCUSSION

The most surprising and significant finding was the extensive distribution of MAP throughout the tissues of cows in which no macroscopic lesions or clinical signs were apparent. It was distributed widely throughout the tissues of 17 of the 21 cows, including three of the four cows that showed no clinical signs of the disease, one of which also had no apparent macroscopic lesions of the disease. The isolates were made in liquid culture and no colony counts were therefore undertaken, and no assessment of the bacterial load in the tissues was made. Although there was little correlation between the presence of lesions associated with Johne's disease and the tissue distribution of MAP there was a correlation between the presence of diarrhoea and the presence of lesions in the large intestine. Lepromatous lesions were detectable only in cases that had extensive granulomatous enteritis and both the large and small intestines were affected by lesions associated with Johne's disease. In most of these cases there were also tuberculoid lesions.

There was no correlation between the extent of the pathological changes due to Johne's disease and the tissue distribution of MAP. In contrast, Pavlik and others (2000) claimed that the organism was widely distributed in the later stages of Johne's disease, as indicated by heavy shedding of the organism in faeces. However, in addition to the draining lymph

TABLE 2: Gross and histological lesions* observed in the intestines of 20 cows from eight herds affected by Johne's disease

Animal†	Proximal jejunum	Mid jejunum	Terminal jejunum	Ileum	Caecum	Proximal colon	Mid colon	Terminal colon
A1	+/b	+/b	+/c	+/c	+/c	+/c	+/c	a/c
A2	+/c	+/c	+/c	+/d	+/d	+/d	+/d	+/d
A3	+/c	+/c	+/c	+/c	+/d	+/c	+/c	a/c
A4	+/c	+/c	+/c	+/c	+/c	+/c	+/c	
A5	+/b	+/b	+/c	+/d	+/c	+/c	a/c	
A6	+/c	+/c	+/c	+/c	+/c	+/c	+/c	
A7	+/b	+/b	+/c	+/c	+/b	+/b	a/b	
B1	+/b	+/b	+/b	+/b				
B2	No visible lesions							
B3			+/b	+/c	+/c	+/c	+/c	a/c
B4	+/d	+/d	+/d	+/d	+/d	+/d	+/d	+/d
B5		+/b	+/b	+/c				
B6	+/d	+/d	+/d	+/d	+/d			
C1	a/c	+/c	+/c	+/c	+/c	a/c		
C2	Chronic mastitis							
C3	Endocarditis							
C4		+/c	+/c	+/c				
D1	No visible lesions							
D2		a/b	a/b	+/b				
D3			a/b	+/b				
D4			+/c	+/c				

* + Gross lesions, a No gross changes but histopathological lesions, b Scattered giant cells in the mucosa, c Tuberculoid lesions, d Lepramatous lesions
† Group A Persistent diarrhoea, Group B Intermittent diarrhoea, Group C No diarrhoea but illthriven, Group D Clinically normal

nodes and other lymphoreticular tissues examined by Pavlik and others (2000), in this study the tonsils and parotid lymph nodes in the head and the prescapular and popliteal lymph nodes were also examined.

The association between the presence of lesions in the large intestine and the development of diarrhoea was strong: 10 of the 11 cows that had lesions in the large intestine also had a history of diarrhoea. In a study of Johne's disease in sheep Clarke and Little (1996) reached similar conclusions and found that diarrhoea tended to occur in sheep with lesions that extended into the large intestine.

The present findings indicate that biopsies of the terminal colon may be of limited use in the confirmation of Johne's disease. Of the 11 animals with lesions of the large intestine only five had detectable lesions in the terminal colon (Table 2). Beurgelt and others (1978) found that only a small proportion of affected cattle had lesions in the terminal colon. They concluded that the liver would be more useful than rectal tissue in confirming a diagnosis of Johne's disease. However, Kreeger (1991) did not regard liver biopsy

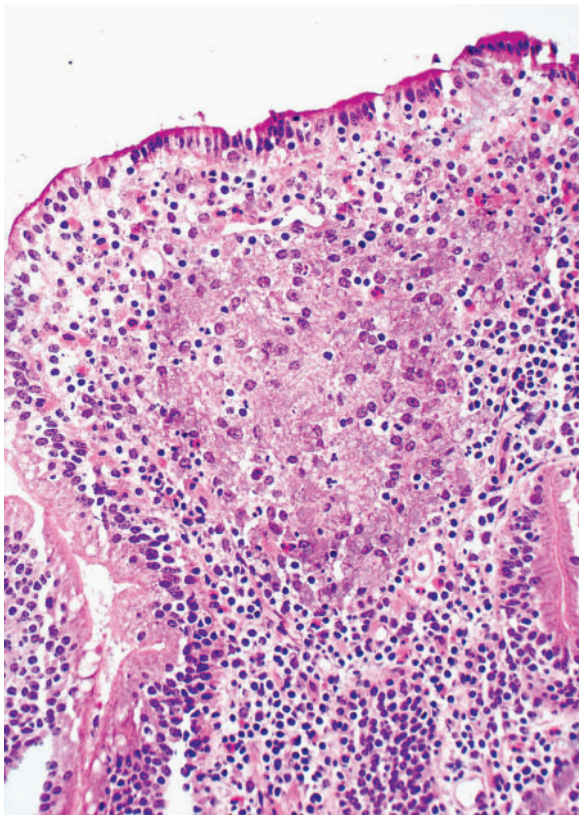

FIG 3: Tuberculoid granulomatous inflammation in the terminal ileum of a cow with Johne's disease. Haematoxylin and eosin. x **400**

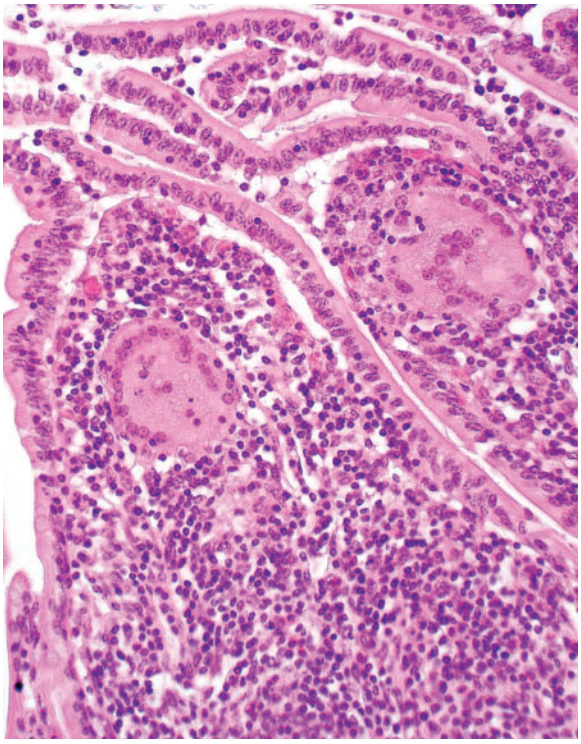

FIG 4: Langhans' giant cells within the villi of the terminal jejunum of a cow with Johne's disease. Haematoxylin and eosin. x **400**

TABLE 3: Distribution of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in the 11 pooled samples of tissues from 21 cows from eight herds affected by Johne's disease

Animal*	Head LNs	Thoracic organs	Terminal ileum	Mesenteric LN	Ileocaecal LN	Caecal contents	Small intestine	Large intestine	Abdominal organs	Prescapular and popliteal LN	Supramammary LN and mammary gland
A1	+	+	+	+	+	+	+	+	+	+	+
A2	CT	+	+	+	+	+	+	+	CT	+	CT
A3	+	+	+	+	+	+	+	+	+	+	+
A4	+	–	+	+	–	+	+	+	–	+	–
A5	+	+	NR	+	NR	+	NR	NR	+	–	NR
A6	–	+	+	+	+	+	–	+	–	+	+
A7	+	+	+	+	+	+	+	+	+	–	–
B1	–	+	+	+	+	+	+	+	–	–	–
B2	–	+	+	+	NR	–	–	–	–	–	–
B3	CT	+	+	NR	+	+	+	+	+	+	NR
B4	+	+	+	+	+	CT	+	+	+	+	+
B5	+	+	+	+	+	+	+	+	+	+	+
B6	CT	–	+	–	+	+	+	–	–	–	–
C1	+	+	+	+	+	NR	+	+	+	+	+
C2	+	–	+	–	+	+	NR	–	NR	NR	NR
C3	–	–	–	–	–	–	–	–	–	+	NR
C4	–	–	–	+	+	+	+	+	–	–	–
D1	+	+	+	+	+	NR	–	+	CT	+	+
D2	–	+	+	+	NR	NR	+	–	–	+	+
D3	–	–	+	–	+	CT	+	–	–	NR	–
D4	+	–	+	+	+	+	+	–	–	+	–

* Group A Persistent diarrhoea, Group B Intermittent diarrhoea, Group C No diarrhoea but illthriven, Group D Clinically normal
LN Lymph node, + MAP cultured, CT Contaminated, – MAP not cultured, NR No result

as an effective means of antemortem diagnosis in cattle. The results of this study support this view because no microgranulomata were found in liver sections taken from any of the 21 animals.

Studies of the blood biochemical changes in Johne's disease in cattle have yielded conflicting results. Patterson and others (1968) found that cattle with clinical Johne's disease had low levels of plasma protein, albumin and globulin, but Kopecky and others (1972) found that the levels of total protein and albumin were within normal limits in such cattle. Hypoproteinaemia, hypoalbuminaemia and normoglobulinaemia have been found to be a consistent finding in sheep with clinical Johne's disease (Scott and others 1995, Jones and Kay 1996). In the present study, most of the cows with clinical Johne's disease had low levels of albumin, but they all had normal levels of globulin. However, the three cows in which no Johne's lesions were detected, for which blood protein levels are available, all had high levels of globulin and significantly higher total protein levels than the other cows. It is possible that normal levels of globulin, together with low or even normal levels of albumin, in an animal with chronic illthrift, either with or without diarrhoea, should increase the suspicion of Johne's disease.

All the cows with Johne's disease for which serological results were available were ELISA-positive, whereas two of the four cows without Johne's lesions were ELISA-negative, suggesting some correlation between the serological results and the development of Johne's lesions. One of these ELISA-negative cows had right-sided endocarditis with multiple abscessation of both lungs, the other had chronic mastitis of all quarters, and MAP was isolated from both of them; in one case (C3) only from the prescapular/popliteal lymph node pool.

The lack of correlation between the clinical signs and the distribution of MAP throughout the body could have public health implications, in view of the concern over the possible links between MAP and Crohn's disease in human beings. This concern has increased since Chiodini and others (1984b) isolated an unclassified *Mycobacterium* species from three patients with Crohn's disease, which was identified by McFadden and others (1987) as MAP. In this study, the three cows which showed no clinical signs but in which MAP was widely distributed (D1, D2 and D4) had high ELISA optical density (OD) values, suggesting that a study of the relationship between the distribution of MAP in tissues and ELISA OD values in a larger number of affected cows would be useful.

Interest in how MAP may enter the food chain has centred on milk because it has been demonstrated that cows with Johne's disease shed MAP in their milk whether or not they show clinical signs (Taylor and others 1981, Sweeny and others 1992). This interest has been heightened since the emergence of evidence that MAP may survive the pasteurisation process (Chiodini and Hermon-Taylor 1993, Hope and others 1996, Millar and others 1996, Grant and others 2002a, b). In this study, MAP was isolated from the supramammary lymph node and mammary gland of eight of 21 cattle, some in each of the four groups, and including two of the four cows that showed no clinical signs. Sweeny and others (1992) isolated MAP from the supramammary lymph nodes of 27 per cent of infected cattle showing no clinical signs and from 11·6 per cent of their milk samples.

The investigations of these 21 cows with suspected Johne's disease found that there was some association between the extent and severity of the lesions and the development of diarrhoea, and that MAP was distributed widely in the tissues of some clinically normal animals. The latter finding may be of public health significance and suggests that further studies of the potential contamination of meat derived from animals infected with MAP should be undertaken.

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