

*Department of Pathology, School of Veterinary Medicine, Shiraz University, PO Box 71345-1731, Shiraz, Iran*

## **The Pathology of Goat Paratuberculosis: Gross and Histopathological Lesions in the Intestines and Mesenteric Lymph Nodes**

A. KHODAKARAM TAFTI<sup>1</sup> and K. RASHIDI

Address of authors: Department of Pathology, School of Veterinary Medicine, Shiraz University, PO Box 71345-1731, Shiraz, Iran; <sup>1</sup>Corresponding author

*With 5 figures and 3 tables*

*(Received for publication May 10, 1999)*

### **Summary**

From a pathological examination of the intestinal tracts of 1590 goats killed at slaughterhouses in the Fars Province of Iran, 59 cases (3.71%) were suspected, on gross examination, of having paratuberculosis. The diagnosis was confirmed by histopathological study and Ziehl–Neelsen staining of direct smears of rectal faeces. On the basis of severity of involvement of the terminal ileum and mesenteric lymph nodes, the microscopic lesions were classified to mild, moderate and severe forms. Caseous necrosis and calcification were observed only in the mesenteric lymph nodes. High numbers of acid-fast organisms were present in the epithelioid macrophages of the intestine but were inapparent or sparse in the mesenteric lymph nodes. On microscopic examination, 13.5% of the suspected animals were found to have paratuberculosis, in comparison with 3.38% by direct faecal smears. In addition, 30.5% and 15.3% of the animals were diagnosed as having eosinophilic enteritis and linguatulus, respectively. These findings stress the importance of a careful histopathological examination of the intestines and mesenteric lymph nodes for the diagnosis of paratuberculosis in goats.

### **Introduction**

Paratuberculosis (Johne's disease) is an economically important infectious disease of high morbidity and low mortality that affects various domestic and wild ruminants. An association of the causative organism with intestinal disease in primates, including man, is also recognized (Chiodini et al., 1984; Van Kruiningen et al., 1986; Clarke, 1997). The infecting organism is *Mycobacterium avium* subspecies *paratuberculosis*, which is a Gram-positive, acid-fast, non-spore-forming bacillus and may cause a persistent intracellular infection of intestinal macrophages and provoke immune and inflammatory reactions in the host tissues (Carlton and Thoen, 1988; Jubb et al., 1993; Clarke, 1997). This disease poses a serious economic problem in many parts of the world, including the whole of Iran. In goats, it was first reported by Twort and Ingram (1912) and in Iran in 1972 (Baharsefat et al., 1972). In Iran, the disease is of most concern in cattle. However, it is being reported with increasing frequency in small ruminants. Estimates of the prevalence of infection in goats or other species of ruminants in Iran are not available. Vaccination of cattle, sheep and goats against paratuberculosis has not been performed in Iran. Since bovine tuberculosis has not been eradicated and vaccination, as a control measure, is only practical in countries with no tuberculosis eradication programme, the interference of vaccination with tuberculin-testing represents a problem.

Because infection with *M. avium paratuberculosis* does not always produce clinical illness, the incidence of the disease, and also its economic importance for small ruminants, especially

goats, has been difficult to establish. The purpose of the study reported here is to describe the gross and histopathological characteristics of paratuberculosis, and its importance and differential diagnosis from other similar diseases in native goats in the Fars Province of Iran.

### Materials and Methods

In this study, the intestines and associated mesenteric lymph nodes from 1590 goats from Fars Assyrian herds killed at slaughterhouses in the Fars province of Iran between February 1998 and September 1998 were carefully examined for thickness and corrugation of the intestinal wall and enlargement of the mesenteric lymph nodes. In those animals grossly suspected of having paratuberculosis, tissue samples were taken from various parts of the intestines, including duodenum, jejunum, ileum, terminal ileum, ileocaecal valve, caecum, colon, rectum and mesenteric lymph nodes, fixed in 10% buffered formalin, embedded in paraffin, sectioned at about 5  $\mu$ m, stained with haematoxylin and eosin (H&E) and studied microscopically. All of the formalin-fixed tissues were also stained with Ziehl-Neelsen's (ZN) acid-fast method. In addition, direct smears of rectal faeces were also prepared and stained using the ZN method. The severity of histopathological changes and amount of acid-fast organisms in different parts of the intestines were scored subjectively on a scale from 0 to 3 where 0 was normal or negative; 1+ was mild changes and small numbers of organisms in the upper part of the lamina propria; 2+ was moderate changes and high numbers of organisms in the upper and deeper parts of the lamina propria; and 3+ was severe changes and high numbers of organisms in the lamina propria and submucosae.

### Results

Of the 1590 cases examined, 59 (3.71%) were suspected of having paratuberculosis on the basis of thickness and corrugation of the intestinal mucosae and enlargement of their mesenteric lymph nodes. The body condition and sex of suspected and positive animals are shown in Table 1.

#### *Gross pathology*

Gross lesions were often insufficient to suggest paratuberculosis (Table 2). In less severe cases, the intestinal mucosae appeared diffusely opaque with a fleshy thickening. The terminal

Table 1. The carcass condition and sex of goats suspected of having and positive for paratuberculosis

Groups	No. examined	Sex		Carcass condition		
		M	F	Normal	Weak	Emaciated
Suspected animals	59	14	45	19	31	9
Positive animals	8	1	7	1	4	3

Table 2. Gross changes in the intestinal tracts of 59 goats suspected of having paratuberculosis

Gross change	Severity		
	Mild	Moderate	Severe
Small intestine			
Mucosal thickening	21	32	6
Mucosal corrugation	7	4	2
Cording of serosal lymphatics	–	1	2
Mesenteric lymph node			
Lymphadenomegaly	11	16	18

ileum and ileocaecal valve had more severe lesions. In more severe cases, a moderate to severe thickening and corrugation of the mucosae of distal parts of the small intestine, especially ileum, which did not disappear on stretching were observed (Fig. 1). Mesenteric lymph nodes were enlarged, oedematous, pale and had some corticomedullary distinction. Lymph nodes associated with the ileocaecal region were most severely affected (Fig. 1). Foci of small granuloma with central calcification were also detectable in some of these nodes. In severe cases, lymphatic vessels were thickened as whitish cords crossing through the mesentery to the lymph nodes.

#### *Histopathology*

On the basis of severity of involvement of the terminal ileum and mesenteric lymph nodes the microscopic lesions were classified as mild, moderate, or severe and compared.

In mild form, the lesions were characterized by focal collections of epithelioid macrophages in the upper part of the lamina propria of the ileal villi. The epithelioid macrophages had a prominent, eosinophilic or foamy cytoplasm with large euchromatic round or ovoid nuclei (Fig. 2). Numerous acid-fast bacilli were visible within these cells by the ZN method. The small foci of epithelioid macrophage aggregations, similar to those described in the ileum, were observed in the paracortical zone of the affected mesenteric lymph nodes. Some of these cells had only small numbers of acid-fast bacilli in their cytoplasm by acid-fast stain.

In moderate form, the lesions were characterized by infiltration and aggregation of epithelioid macrophages and some lymphocytes, eosinophils and plasma cells in the upper and deeper zones of the lamina propria of the ileum and eventually, of the whole lamina propria. The villi were mildly atrophic and blunt and the crypts of Lieberkühn glands were compressed to some extent. Many of the epithelioid macrophages were seen to be packed with acid-fast bacilli using the ZN method. Remarkable infiltration of lymphocytes was observed in the upper parts of the submucosae, particularly around the lymphoid vessels. Various sized epithelioid



Fig. 1. Ileum and mesenteric lymph node from a goat with paratuberculosis. The intestinal surface is thickened and corrugated and the mesenteric lymph node is enlarged and shows granulomatous inflammation.

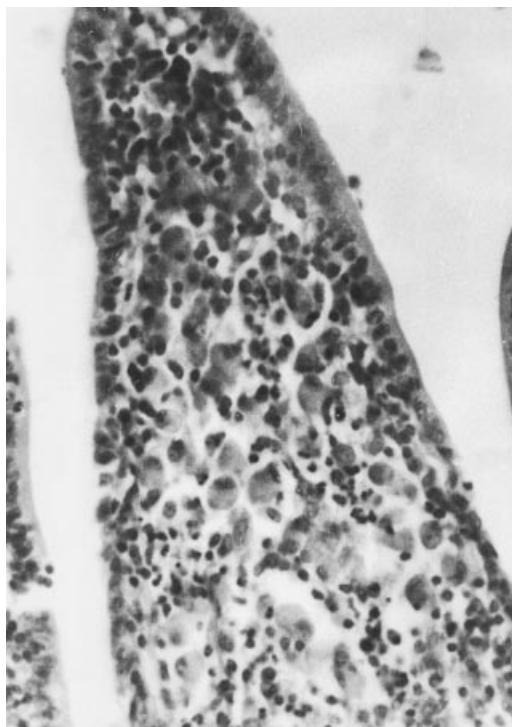


Fig. 2. Ileum from a goat with paratuberculosis; mild lesion. Focal aggregations of epithelioid macrophages in the lamina propria of the villous head. H & E,  $\times 300$ .

macrophage microgranulomas were present in the paracortical zone and subcapsular sinuses of mesenteric lymph nodes (sinus histiocytosis) (Fig. 3). They contained only a few acid-fast organisms.

In severe form, the lesions were characterized by infiltration and aggregation of many epithelioid macrophages, a few giant cells and numerous lymphocytes, plasma cells, eosinophils and sometimes a few neutrophils in the mucosae, submucosae and even muscle tunica and serosa. The whole lamina propria was invaded by massive numbers of epithelioid macrophages which compressed the crypts of Lieberkühn glands and the villi were severely atrophic and blunted. The ZN method showed many acid-fast bacilli within epithelioid macrophages in the affected areas. Lymphangitis was more common and was characterized by aggregations of lymphocytes, plasma cells and a few macrophages around the lymph vessels in the submucosae and particularly serosa. In the mesenteric lymph nodes, confluent aggregates of epithelioid macrophages and Langhans' giant cells replaced the subcapsular and cortical areas (Fig. 4). Focal to diffuse areas of caseous necrosis, with or without central calcification, were also observed. A few epithelioid macrophages and giant cells had only small numbers of acid-fast bacilli which were not free in the necrotic areas.

Granulomatous lymphangitis was observed in the mesenteric lymph nodes (Fig. 5) that did not contain acid-fast bacilli. In one case, a severe form of paratuberculosis and linguatuberculosis were observed occurring concurrently in mesenteric lymph nodes.

Of the grossly suspected cases, examination of ZN-stained direct faecal smears showed two (3.38%) to be positive, with the presence of clumps of *M. avium paratuberculosis*. The severity of histopathological changes and amount of acid-fast organisms in different parts of the intestines are shown in Table 3.

Microscopically, 30.5% of the grossly suspected animals had lesions of eosinophilic

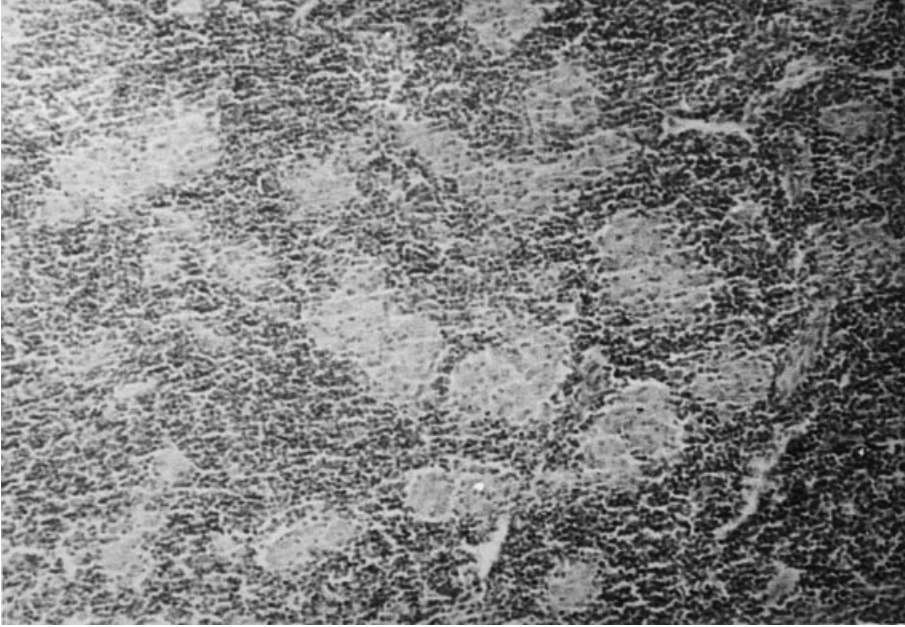


Fig. 3. Paratuberculosis in goat: various size epithelioid macrophage microgranulomas in the cortical zone of mesenteric lymph node. H & E,  $\times 84$ .

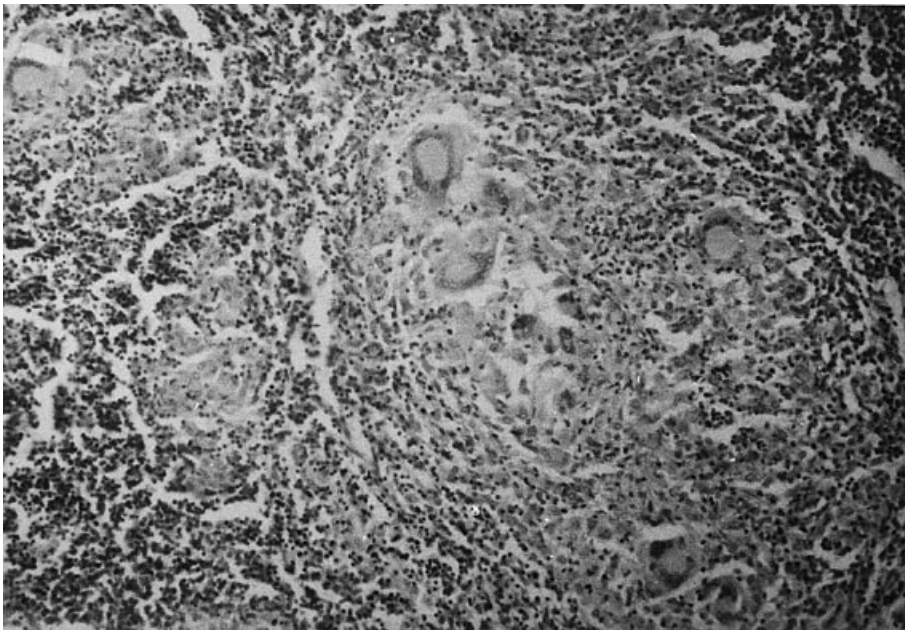


Fig. 4. Paratuberculosis in goat: confluent aggregate of epithelioid macrophages and Langhans' giant cells replaced the cortical areas of mesenteric lymph node. H & E,  $\times 140$ .

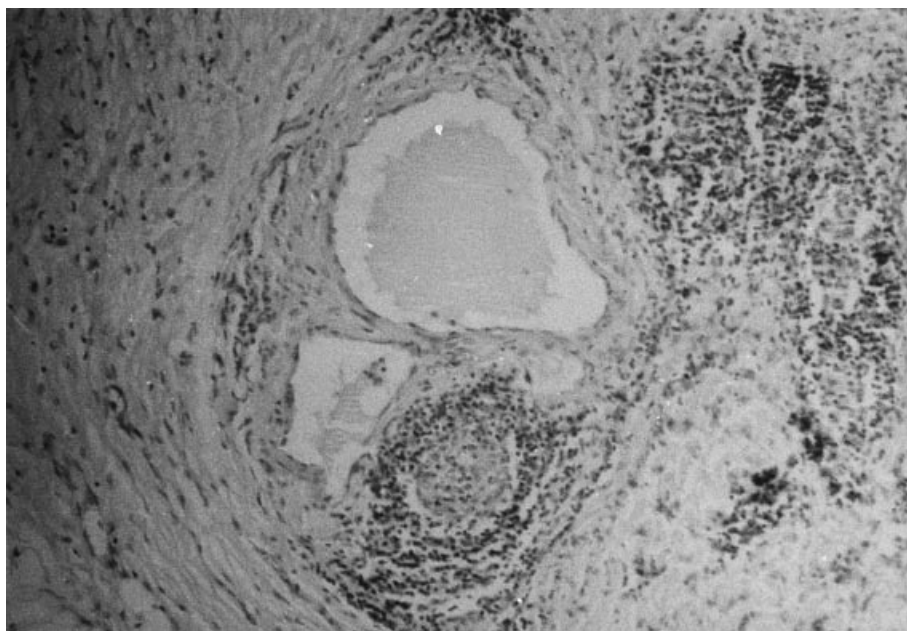


Fig. 5. Paratuberculosis in goat: granulomatous lymphangitis with focal aggregation of epithelioid macrophages and lymphocytes in the mesenteric lymph node. H & E,  $\times 233$ .

Table 3. The histopathological changes and amount of acid-fast bacilli in different parts of intestines of goats affected with paratuberculosis

Case	Duoden.	Jejun.	Ileum	Ileocaec.	Caecum	Colon
1	–	–	1+	1+	–	–
2	–	–	2+	2+	1+	–
3	–	1+	3+	3+	1+	1+
4	–	–	2+	1+	–	–
5	–	–	1+	2+	–	–
6	–	–	2+	2+	–	–
7	–	1+	3+	3+	1+	1+
8	–	–	1+	1+	–	–

Duoden., duodenum; Jejun., jejunum; Ileocaec., ileocaecal valve; –, Normal or negative; 1+, mild changes and small numbers of organisms in the upper part of the lamina propria; 2+, moderate changes and high numbers of organisms in the upper and deeper parts of the lamina propria; 3+, severe changes and high numbers of organisms in the lamina propria and submucosae.

enteritis, sometimes with the presence of nematode sections in the mucosae of intestines, and 15.3% had lesions due to migration of *Linguatula* larvae in the mesenteric lymph nodes.

### Discussion

Paratuberculosis in goats is probably more prevalent in the world than the current literature indicates. Unlike cattle, goats do not usually show signs of diarrhoea and because of the gradual onset of weakness and emaciation, the disease is overlooked in most cases. Studies on its

prevalence, based on various methods such as gross and histological examinations, organism culture, serology and the demonstration of mycobacterial DNA in faeces or tissues of cattle, have revealed evidence of infection up to 5% in UK and 18% in the USA (Chiodini et al., 1984; Braun et al., 1990; Clarke, 1997).

Although there are no records of its prevalence in small ruminants in Iran, our observations on the basis of case files from the Department of Veterinary Pathology, School of Veterinary Medicine from 1993 to 1998 showed an increasing trend in its incidence. The gradually increasing infection rate is supported by the annual reports from the veterinary organization and also by the reports of veterinary practitioners in local journals. In a report from Norway, various degrees of thickening and marked corrugation of the mucosae of the small intestine were found in about 40% and 7%, respectively, of the 273 infected goats that were examined (Fodstad and Gunnarsson, 1979). Kumar et al. (1988) reported an incidence of paratuberculosis of 8.76% among goats in India based on a retrospective study on necropsy data from a 15-year period (1972–86). In their study, female goats suffered more than males. The present study also showed the disease in females more than males. This higher incidence of paratuberculosis in females compared to males in this study might be a result of the increased abundance of female goats among those slaughtered in Fars Province rather than a result of increased female susceptibility due to the reproductive stresses which females have during their lifetime but it needs further elucidation. Also, the increased incidence may be related to the organism *M. avium paratuberculosis* being more pathogenic for the female, as was *M. tuberculosis* in mice (Youmans and Youmans, 1959; Gezon et al., 1988; Kumar et al., 1988).

The pathological findings reported here were similar to those reported earlier for paratuberculosis in goats and sheep (Stamp and Watt, 1954; Moser, 1982; Morin, 1982; Sweeny et al., 1984; Gezon et al., 1988; Carrigan and Seaman, 1990; Clarke and Little, 1996). The present study reveals gross lesions in the intestine limited to a mild to severe thickening. This is similar to the findings of Williams and Sparker (1979), but contrasts with the findings of Gezon et al. (1988) and with lesions produced in some experimentally infected sheep (Nisbet et al., 1962; Kluge et al., 1968). Numerous giant cells were seen in the mesenteric lymph nodes of goats in our study. This finding agrees with the observation of Gezon et al. (1988), but contrasts with the findings of Moser (1982). In this investigation, caseous necrosis and calcification were not seen in the intestines of goats. This is similar to the findings of Moser (1982), Ullrich et al. (1982) and Gezon et al. (1988), but contrasts with the findings of Morin (1982). The present study showed caseous necrosis and calcification in the mesenteric lymph nodes. This finding agrees with the observations of Morin (1982), Moser (1982), Ullrich et al. (1982), Thomas (1983) and Gezon et al. (1988). Corrugation of the mucosae of distal small intestine could sometimes have been missed. In addition, many other agents, such as parasites, can induce similar lesions; indeed, in our study most of the suspected animals were also affected by parasitic infections. Gross lesions in the mesenteric lymph nodes were limited to enlargement, with or without areas of necrosis and calcification. These lesions could have been seen in other diseases, as in the present study linguatulosis was more common.

The histopathological lesions of paratuberculosis in sheep have been reported in detail and various classification systems have been proposed (Stamp and Watt, 1954; Rajya and Singh, 1961; Reddy et al., 1984; Carrigan and Seaman, 1990; Perez et al., 1992, 1996; Clarke and Little, 1996) but they have not been classified specifically in goats. The histopathological lesions in our study in goats were somewhat different to those seen by some other workers. Giant cells were rarely seen by Power et al. (1993) who, working on paratuberculosis in farmed red deer, reported a granulomatous enteritis but did not find necrotic tubercles in the intestine.

However, nodular foci of caseous necrosis and calcification have been described, particularly in the mesenteric lymph nodes of goats (Collins et al., 1984). There is some evidence that subspecies other than *M. avium paratuberculosis*, such as *M. avium silvaticum*, may be implicated in many caprine cases, possibly accounting for the particularly prominent focal tuberculous lesions (Collins et al., 1984; Thorel et al., 1990).

In the present study, acid-fast organisms were usually inapparent or sparse in the mesenteric lymph nodes. This finding agrees with that of Clarke and Little (1996). The apparent absence of acid-fast mycobacteria in many tuberculoid lesions stained by the ZN technique

may reflect sparsity of organisms, change in morphology, or poor sensitivity of staining (Clarke, 1997).

In our study, as shown in Table 2, there were most severe histological changes in the ileum and ileocaecal valve, with the presence of high numbers of acid-fast organisms. Also, the mesenteric lymph nodes were found to be the main lesion site with presence of small numbers of acid-fast bacilli. On the basis of our experience, in goats, the tissues to be selected for pathological diagnosis of paratuberculosis are the terminal ileum, ileocaecal valve and associated mesenteric lymph nodes. It is very easy to find bacilli in the ileum and ileocaecal valve and these are recommended for future studies. However, this study emphasizes the importance of a careful histopathological examination of the intestines and mesenteric lymph nodes for diagnosis of paratuberculosis in goats.

### Acknowledgements

We would like to thank Professor Dr S.N.S. Gaur, Mrs Roodaki and Mr Khatami for their help and Mrs Farasat for typing the manuscript.

### References

- Baharsefat, M., A. R. Amjadi, P. Ahourai, B. Yamini, F. Entessar, and H. Hedayati, 1972: Paratuberculosis in sheep and goat in Iran. Epidemiological, pathological features and laboratory diagnosis. Arch. Instit. Razi **24**, 49–61.
- Braun, R. K., C. D. Buergelt, R. C. Littell, S. B. Linda, and J. R. Simpson, 1990: Use of an enzyme-linked immunoabsorbent assay to estimate prevalence of paratuberculosis in cattle in Florida. J. Am. Vet. Med. Assoc. **196**, 1251–1258.
- Carlton, L. G., and C. D. Thoen, 1988: Pathogenesis of Bacterial Infections in Animals. 2nd edn., pp. 26–34. Iowa State University Press. Ames.
- Carrigan, M. J., and J. T. Seaman, 1990: The pathology of Johne's disease in sheep. Aust. Vet. J. **67**, 47–50.
- Chiodini, R. J., H. J. Van Kruiningen, and R. S. Merkal, 1984: Ruminant paratuberculosis (Johne's disease): The current status and future prospects. Cornell Vet. **74**, 218–262.
- Clarke, C. J., 1997: The pathology and pathogenesis of paratuberculosis in ruminants and other species. J. Comp. Pathol. **116**, 217–261.
- Clarke, C. J., and D. Little, 1996: The pathology of ovine paratuberculosis: gross and histological changes in the intestine and other tissues. J. Comp. Pathol. **114**, 419–437.
- Collins, P., D. C. Davies, and P. R. J. Matthews, 1984: Mycobacterial infection in goats: diagnosis and pathogenicity of the organism. Br. Vet. J. **140**, 196–201.
- Fodstad, F. H., and E. Gunnarsson, 1979: Postmortem examination in the diagnosis of Johne's disease in goats. Acta Vet. Scand. **20**, 157–167.
- Gezon, M., H. D. Bither, H. C. Gibbs, E. J. Acker, A. Hanson, and J. K. Thompson, 1988: Identification and control of paratuberculosis in large goat herd. Am. J. Vet. Res. **49**, 1817–1823.
- Jubb, K. V. F., and P. C. Kennedy, N. Palmer, 1993: Pathology of Domestic Animals. 4th Edn, Vol. 2, pp. 247–252. Academic Press, Inc, San Diego, CA.
- Kluge, J. P., R. S. Merkal, W. S. Monlux, A. B. Larsen, K. E. Kobecky, F. K. Ramsey, and R. P. Lehmann, 1968: Experimental paratuberculosis in sheep after oral, intratracheal, or intravenous inoculation: Lesions and demonstration of etiologic agent. Am. J. Vet. Res. **29**, 953–962.
- Kumar, R., and M. C. Prasad, O. P. Paliwal, 1988: Paratuberculosis in goats. A retrospective study. Indian Vet. J. **65**, 582–584.
- Morin, M., 1982: Johne's disease (Paratuberculosis) in goats: a report of eight cases in Quebec. Can. Vet. J. **23**, 55–58.
- Moser, C. L., 1982: Johne's disease (Paratuberculosis) in a goat. Can. Vet. J. **23**, 63–66.
- Nisbet, D. I., N. J. L. Gilmour, and J. G. Brotherton, 1962: Quantitative studies of *Mycobacterium Johnei* in tissues of sheep. J. Comp. Pathol. **72**, 80–91.
- Perez, V., J. F. Garcia Martin, and J. J. Badiola, 1992: Pathological features in sheep naturally infected with *M. paratuberculosis*. Med. Vet. **9**, 20–22.
- Perez, V., J. F. Garcia Martin, and J. J. Badiola, 1996: Description and classification of different types of lesion associated with natural paratuberculosis infection in sheep. J. Comp. Pathol. **114**, 101–122.



- Power, S. B., J. Haagsma, and D. P. Smyth, 1993: Paratuberculosis in farmed red deer (*Cervus elaphus*) in Ireland. *Vet. Rec.* **132**, 213–216.
- Rajya, B. S., and C. M. Singh, 1961: Studies on the pathology of Johne's disease in sheep III. Pathologic changes in sheep with naturally occurring infections. *Am. J. Vet. Res.* **22**, 189–203.
- Reddy, K. P., P. K. Sriraman, N. R. Gopal Naidu, and P. Rama Rao, 1984: Pathology of Johne's disease in sheep. *Indian Vet. J.* **61**, 179–184.
- Stamp, J. T., and J. A. Watt, 1954: Johne's disease in sheep. *J. Comp. Pathol.* **64**, 26–40.
- Sweeny, R. W., T. Diver, and D. Show, 1984: Paratuberculosis in a ram. *J. Comp. Pathol.* **185**, 444–445.
- Thomas, G. W., 1983: Paratuberculosis in large goat herd. *Vet. Rec.* **113**, 346–466.
- Twort, F. W., and G. L. Y. Ingram, 1912: A method for isolating and cultivating *Mycobacterium enteritidis choronicae pseudotuberculosis bovis johne* and some experiments on the preparation of a diagnostic vaccine for pseudotuberculosis enteritis of bovines. *Proc. Royal Soc. London* **84**, 517–543.
- Thorel, M. F., M. Krichevsky, and V. V. Levy-Frebault, 1990: Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of *Mycobacterium avium*, and description of *Mycobacterium avium* subsp. *avium* subsp. *nov.*, *Mycobacterium avium* subsp. *paratuberculosis* subsp. *nov.*, and *Mycobacterium avium* subsp. *silvaticum* subsp. *nov.* *Int. J. Syst. Bacteriol.* **40**, 254–260.
- Ullrich, N. A., S. Grumbein, and B. Coles, 1982: Paratuberculosis (Johne's disease) in goats. *Vet. Med. Sm. Anim. Clin.* **77**, 1101–1104.
- Van Kruiningen, H. J., R. J. Chiodini, W. R. Thayer, J. A. Coutu, R., S. Merkal, and P. L. Runnels, 1986: Experimental disease in infant goats induced by a *Mycobacterium* isolated from a patient with crohn's disease. *Diges. Dis. Sci.* **31**, 1351–1360.
- Williams, E. S., and T. Sparker, 1979: Paratuberculosis in Bighorn sheep and a Rocky Mountain goat in Colorado. *J. Wildlife Dis.* **15**, 221–227.
- Youmans, G. P., and A. S. Youmans, 1959: The relationship of sex to the susceptibility of normal and immunized mice to tuberculosis. *Am. Rev. Resp. Dis.* **80**, 750–752.