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FIRST DIAGNOSIS OF SHEEP PARATUBERCULOSIS IN SERBIA

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Sheep paratuberculosis was diagnosed recently (1995) in Serbia, for the first time. The disease occurred sporadically in a flock of Ile de France sheep and led to a 3-4% loss annually. The disease was characterised by occasional diarrhoea, progressive weight loss, emaciation and death.

Postmortem examination of dead or sacrificed animals revealed cachexia and hydrops of body cavities. In addition, changes in the intestines and associated lymph nodes were observed. They were particularly distinct in the ileum which was thickened, especially at the end, thus resembling the oesophagus.

Under the microscope, the intestine, especially the ileum, showed thickening of lamina propria which was diffusely infiltrated by macrophages and a small number of lymphocytes and neutrophils. The same infiltrate was noted in the intestinal submucosa and lymph nodes. Ziehl-Neelsen staining of the tissue section revealed a large number of acid-fast, short rods intracellularly in the macrophages, thereby confirming the initial diagnosis with certainty.

The described changes, with regard to their character, were of the lepromatous ("multibacillary") type, alterations of the tuberculoid ("paucibacillary") type were not seen in any animals.

Key words: paratuberculosis, sheep, intestine, histopathology, lesions

INTRODUCTION

Paratuberculosis or Johne's disease is an infective disorder of the digestive tract of animals, especially of ruminants, that is caused by Mycobacterium avium subspecies paratuberculosis. The clinical and pathoanatomical picture of this disease was first described during the 19th century. In 1985 the English investigators H. A. Johne and L. Forthingham demonstrated a connection between

cattle enteritis and the presence of acid-fast microorganisms in sections of the intestinal mucosa, which is why this disease was called Johne's disease (Cocito et al., 1994).

Sheep paratuberculosis has been described in various regions worldwide and today Johne's disease is one of the most widespread bacterial diseases of domestic animals which causes big economic losses. For example, the yearly losses due to this disease have been estimated to exceed \ 1,5 billion in the United States (Cocito et al., 1994).

Data on autbreaks of paratuberculosis are scarce for the territory covered by the former Republic of Yugoslavia. The exception is the report of Vuković (1948) about the occurrence Johne's disease in Bosnia, but he neither mentioned the time nor the place where the disease was diagnosed. Also, there is a recent report on the diagnosis of sheep paratuberculosis in Slovenia (Pogacnik et al., 1995). In Serbia there have been no reports about outbreaks of paratuberculosis till now when we recorded the disease in 1995, for the first time, in a flock of Ile de France sheep.

It this paper we will describe our experience with regard to this disease.

MATERIALS AND METHODS

Ten adult females Ile de France sheep, 2-6 years of age, were examined. The sheep originated from a single flock which contained 300-400 breeding sheep, depending on the year. The flock history for a few years back showed an annual loss of about 3-4% of the adult animals. The disease was characterised by occasional diarrhoea, progressive loss of physical fitness, emaciation and death. Therapy with vitamins, antibiotics and antiparasiticides produced no effect. Clinical signs in the affected animals usually appeared at the end of winter and beginning of spring, after the lammbing season.

The materials originated from dead (n=3) or culled (intravenous barbiturate administration) animals in the final stage of the disease (n=7). After a detailed gross examination samples of the jejunum, ileum, caecum, ileocaecal and mesenterial lymph nodes, spleen, liver, kidney and lung were collected. The fresh samples of tissue were fixed in neutral formalin and processed by routine methods. Paraffin sections, (thick 8-10 mm), were stained with haematoxylin and eosin (HE), and according to the Ziehl Neelsen (ZN) method to detect acid-fast bacteria.

We would like to point out, that our investigation included only animals where acid-fast bacteria were demonstrated in sections of intestinal mucosa by the ZN mmethod.

RESULTS

Necropsy and gross lesions. All animals, dead and sacrificed, were emaciated. In three animals loss of wool was noticed from one part of the back, and in four animals the anus and the surrounding wool were stained by faeces.

Faeces in the latter animals were pasty or liquid whereas other animals had firm, pelleted faeces. All animals had subcutaneous oedema and hydrops of body cavities.

During the macroscopic examination special attention was paid to the appearance of the intestines.In seven animals there was thickening of the walls of the intestine, particularly of the ileum. The terminal ileum was thickened and rigid, resembling the oesophagus. The ileal mucosa was slightly granular; white to yellow in colour and moderately transversely wrinkled. Gross lesions in the proximal (jejunum) and distal (caecum and colon) parts of the intestine were less prominent. In three animals the changes in the intestines were very mild and could not be easily noticed, even in the ileum. In five animals the ileal serosa was oedematous with prominent cording of th serosal lymphatic ducts. In all animals mesenteric and ileocaecal lymph nodes were enlarged, whitish gray, oedematous at the cut surfaces without a clear distinction between the cortex and the medulla. Caseation necrosis and calcification were not observed macroscopisally in any animal. The spleen, liver, kidneys and lungs were grossly normal in all sheep.

Histopathology. The most severe changes in all the examined animals were consistently located in the ileum and involved all the layers of the intestinal wall. The lamina propria mucosa of the ileum was thickened due to a cell infiltrate which mainly consisted of macrophage-epitheloid cells with abundant, weakly eosinaphilic cytoplasm and large vesicular nucleus. A small number of cells were lymphocytes, eosinophils and neutrophils. In two animals the infiltrate contained a greater number of neutrophils which were focally distributed amongst the macrophages. Multinucleate Langerhans giant cells were not observed in any cases. There was severe villous atrophy and fusion, resulting in a substantially flattened mucosal surface (Figure 1). The number of crypts of epithelial glands was lower and some of them were distended and filled with cellular debris (Figure 1b).

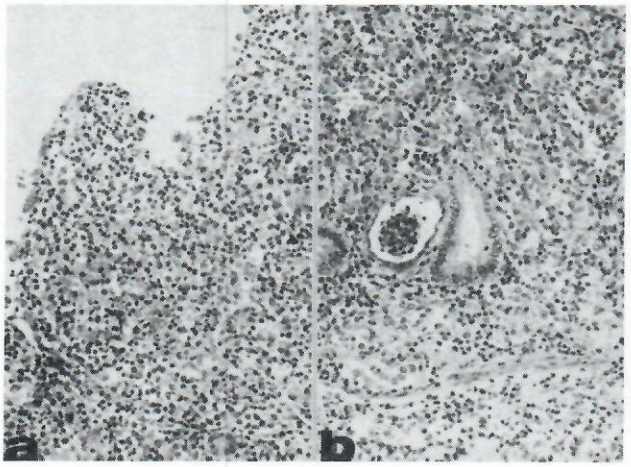

Figure 1. Marked infiltration into the lamina propria by macrophages with abundant cytoplasm. a) Villous atrophy and fusion b) Surviving crypt epithelial glands are distended. HE. x 200.

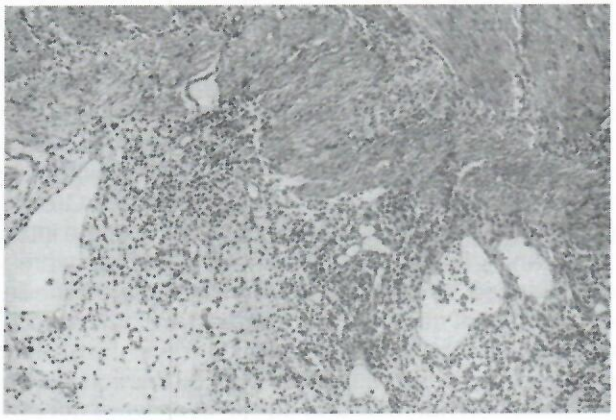

Figure 2. Infiltration between muscle band layers and serosa by macrophages and lymphocytes. Lymphatic vessels are dilated. HE. x 100.

A cellular infiltrate of similar composition was also seen in the ileal submucosa in all animals. It had a focal distribution and consisted mainly of macrophages and a small number of lymphocytes. The submucosa, also, showed lymphatic dilation and perilymphatic lymphoid cell accumulation. Lymphoid cell infiltration was also seen in the muscle band layers, especially between circular and longitudinal bands (Figure 2).

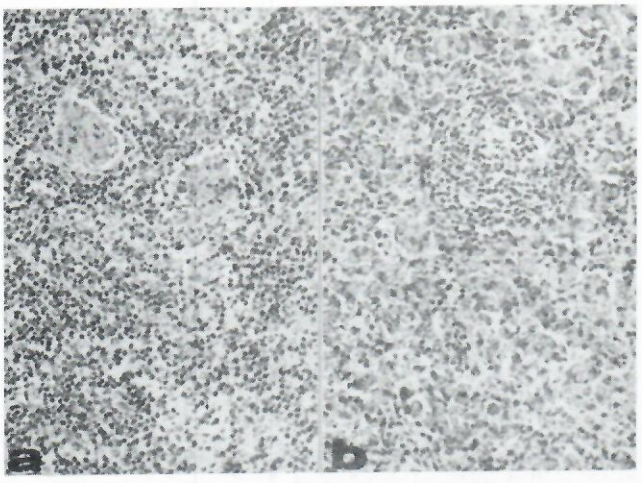

Figure 3. a) Granulomas in mesenteric lymph node cortex. HE. x 20. b) Diffuse infiltration by macrophages in mesenteric lymph node cortex. HE. x 200.

In the serosa lymphatic dilatation with thickening of the walls and perilymphatic and perivascular lymphocyte aggregation were recorded. Changes in the other parts of the intestines (jejunum, caecum and colon) were similar to those in the ileum, but of a weaker intesity.

Both mesenteric and ileocaecal lymph nodes were hyperplastic with an evident lymphoid proliferation. Macrophages were present in the cortex as a diffuse infiltration or formed multifocal small granulomata (Figure 3). A large number of microphages was also noted in the subcapsular sinuses.

After staining of the sections of the ileum by the ZN method a large number of macrophages containing acid-fast rods in their cytoplasm was observed to infiltrate in the lamina propria in all animals. The bacteria were numerous in each macrophage (Figure 4). The presence of acid-fast organisms was closely related to the extent of epitheloid cell infiltration.

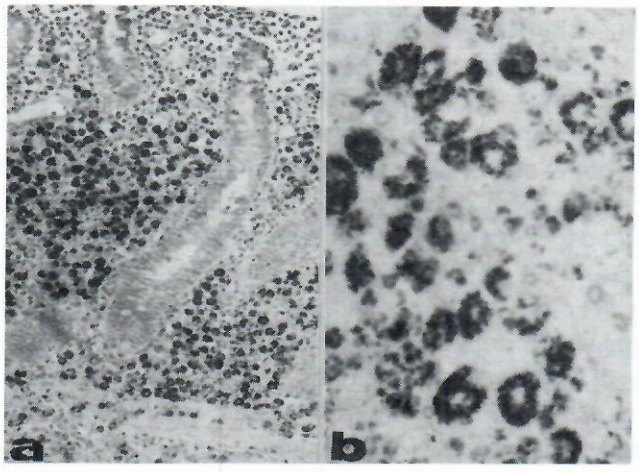

Figure 4. a) Numerous acid fast rods pack the cytoplasm of macrophages infiltrating the intestinal lamina propria and submucosa. ZN. x 200. b) Detail. ZN. x 400.

Acid-fast rods could also be seen in the macrophages infiltrating submucosa and lumph nodes, but only in small numbers.

In the spleens, livers, kidneys and lungs significant histological changes were not observed.

DISCUSSION

Paratuberculosis was diagnosed on a farm in Serbia in 10 sheep, on the basis of the clinical picture of the disease, pathohistologically confirmed granulomatous enteritis and regional lymphadenitis, as well as the detection of

acid-fast bacilli in the tissues. All animals manifested the same clinical signs of the disease (a progressive loss of weight accompanied by occasional diarrhoea) although macroscopic findings in three animals on postmortem examination failed to be clearly discernible. As in other reports (Carrigan and Seaman, 1990; Clarke and Litle, 1996), in our case too, the microscopic changes on the ileum were unmistakable. This finding is easily explainable because the ileum, i. e. the epithelium which covers the lymphatic tissue of this portion of the small intestine, plays an important role in the pathogenesis of paratuberculosis, being the entrance door for its causative agent (Momotani et al., 1988; Cocito et al., 1994). Yellow orange staining of the intestinal mucosa reported by certain authors (Clarke and Little, 1996; Jubb et al., 1993) was not observed. Since the tissue staining is due to the property of some strains of M. a. paratuberculosis to impart pigment (Jubb et al., 1993.), we believe that such strains were missing in the investigated animals. This, however, did not affect the nature of the incured changes, because the pigmented and non-pigmented strains of M. a. paratuberculosis are pathogenetically similar (Clark and Little, 1996).

Microscopic changes were expressed in all animals to an extent that even macroscopy negative sheep showed intensive infiltration by macrophages, which contained a large number of acid-fast bacteria. Disagreement between macroscopic and microscopic findings in sheep paratuberculosis was reported by Carrigan and Seaman (1990) who found 36% of their cases to be macroscopy-negative. Perez et al., (1996) proposed that the presence or absence of macroscopic changes would depend on the type of histological changes. The basic characteristics of the histological changes consisted of an intensive infiltration of the intestinal mucosa propria by a large number of macrophages which, in turn, contain a large number of acid-fast bacteria, and changes resembling the so-called lepromatous type of disease. These alterations found in the course of the study correspond to the changes designated as type I by Rajya and Singh, (1961) or type IIIb (Perez et al., 1996) or multibacillary type (Clarke and Little, 1996). Tuberculoid type changes designated by the same authors as type II, IIIc orpaucibacillary type, respectively, were not observed in any of the studied cases.Carrigan and Seaman, (1990) described changes which were identical, in the majority of cases, to those observed in our study. Multinuclear giant cells were not seen in any section of the intestine or lymph nodes. This finding is in agreement with those of other authors (Clarke and Little, 1996; Perez et al., 1996) on the scarceness of giant cells in the lepromatous form of paratuberculosis which appear in a greater number in the tuberculoid type of the disease. In addition to its characteristic pathohistological picture, the lepromatous type is also characterized by the presence of a large number of acid-fast bacteria, by a

strong, but ineffective, humoral immune response and a weak cellular response, which indicate the weak resistance of the animals (Clark and Little, 1996). The pathogenesis of such a granulomatous inflammation is greatly helped by the lymphokines TNF alpha, IL-1 and IL-6, which increase in the changed tissues (Alzuherri et al., 1996).

Our study was carried out on animals with a clear clinical pisture, i. e. poorly resistant animals, which explains the observed microscopic changes. All the diseased animals were aged two or more years, thereby confirming that paratuberculosis affects adult animals, although a majority of them contracts the disease in the first months after birth (Larsen et al., 1975; Cocito et al., 1994; Perez et al., 1996). The appearance of the disease symptoms will depend on the immunological status of the animal (Bendixen, 1978; Gilmour et al., 1978). On our farm the greatest nummber of clinically diseased animals was noticed at the end of winter and in spring which is easily explained by failing resistance, due to inadequate feeding during the winter at the time of pregnancy or intensive lactation after lambing. This is further supported by the fact that all the diseased animals were females and all had already lambed. However, the predominance of females over males by 10:1 on the farm should not be neglected.

Paratuberculosis was diagnosed on the basis of a typical pathohistological picture and the detection of acid-fast bacteria using the staining method of Ziehl-Neelsen. Other authors (Clarke and Little, 1996; Perez et al., 1996) reported that the Ziehl-Neelsen method proved to be 100% reliable for the detection of mycobacteria characteristic of the multibacillary type of changes. In the paucibacillary type of changes it is much harder to detect bacteria. Somewhat better results have been obtained using immunohistochemical methods, anti-M.a. paratuberculosis antibodies or macrophage markers (Perez et al., 1996). On the other hand, data are controversial on how valuable the method of cultivating the causative agent may be. Most of the authors (Carrigan and Seaman, 1990; Collins et al., 1993; Jubb et al., 1993) agree that the strains of M. a. paratuberculosis which cause sheep paratuberculosis are difficult to raise in culture. Perez et al. (1996) were much more successful using the Levenstein-Jansen substrate. The discrepancy may be due to both the procedure and the strain used. We have also set up cultures of the causative agent isolated from ileum tissue and the faeces but the data are not yet available.

In conclusion, we believe that the paratuberculosis diagnosis should be taken seriously, especially where the disease breaks out for the first time. Adequate steps can then be taken in due course to reduce dissemination of paratuberculosis in order to avoid vast economic losses. When assessing its possible impact on health and breeding of animals, we should not neglect the

fact that Mycobacterium avium paratuberculosis has also been isolated in some cases of Crohn's disease in humans (Sanderson et al., 1992, Moss et al., 1992).

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PRVA DIJAGNOSTIKA OVČIJE PARATUBERKULOZE U SRBIJI

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SADRŽAJ

U Srbiji je 1995. godine otkrivena po prvi put paratuberkuloza ovaca. Bolest se javila sporadično u jednom stadu ovaca rase Ile de France, nanoseći gubitak 3-4% grla godišnje. Oboljenje se manifestovalo povremenim prolivima, progresivnim gubitkom telesne mase i uginućem životnja.

Na obdukciji uginulih ili žrtvovanih životinja uočavana je kaheksija i hidrops telesnih duplji, a mogle su se zapaziti ipromene na crevima i pripadajućim limfnim čvorovima. Promene su posebno bile uočljive na ileumu, koji je zadebljao, naročito njegov završni deo, tako da je pokazivao cevast izgled sličan jednjaku.

Mikroskopskim pregledom creva, naročito ileuma, uočeno je zadebljanje proprije mukoze koja je bila difuzno infiltrovana makrofagama, manjim brojem limfocita i granulocita. Isti infiltrat zapažen je u submukozi i limfnim čvorovima, naročito u njihovom kortikalnom delu. U isečcima tkiva bojenim metodom po Ziehl Neelsen-u, intracelularno u makkrofagama zapažen je veliki broj mikobakterija u vidu crveno obojenih kratkih štapića, čime je dijagnoza definitivno potvrđena.

Opisane promene po svom karakteru pripadale su lepromatoznom (multibacilarnom) tipu, dok promene tuberkuloidnog tipa nisu zapažene ni kod jedne životinje.