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Concurrent occurrence of visceral linguatulosis and paratuberculosis in alpine cross goats (*Capra hircus*)

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ABSTRACT

Concurrent visceral linguatulosis and paratuberculosis was diagnosed in five Alpine Cross goats (*Capra hircus*). Severe, gross and histopathological changes wereobserved, with the occurrence of multibacillary paratuberculosis and parasite-induced damage. The PCR-based technique was employed, using IS 900, to confirm paratuberculosis, and light, stereo- and scanning electron-microscopy were carried out to study the third-instar larvae of *Linguatula serrata*, Frohlich, 1789. The morphological changes were mainly in the intestines and mesenteric lymph nodes and paratuberculosis-associated lesions were principally of a diffuse multibacillary type, with a severe granulomatous reaction, consisting of macrophages laden with large numbers of acid-fast bacilli and variously sized cyst-like spaces in the lymph nodes, histologically associated with the moth-eaten appearance of the parenchyma. Severely oedematous and haemorrhagic lymph nodes, having areas of calcification with profuse numbers of *Mycobacterium avium* subsp. *paratuberculosis* (Map), seemed to be characteristic of the concurrent occurrences of the diseases. The present investigation suggests that the parasite, being lymphovorous, might predispose to the multibacillary form of paratuberculosis.

Key words: linguatulosis, paratuberculosis, pathology, goats

Introduction

Linguatula serrata is a complete parasite, highly specialised in endoparasitism, with the adults residing in the upper respiratory tracts of carnivore mammals, inducing parasitic rhinitis (AKYOL et al., 1995) and the larvae and nymph migrating and encysting in the various visceral organs of herbivores. Omnivorous mammals, including humans, may act

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either as final or intermediate hosts. Therefore the presence of this infection in carnivores or herbivores is potentially zoonotic, causing visceral and nasopharyngeal linguatulosis or 'Halazoun Syndrome' in humans (LAZO et al., 1999). Reports of its occurrence in India and Bangladesh have been documented and its occurrence in the Kashmir valley has been recently established (MIR et al., 2004). The concurrent occurrence of this parasite with paratuberculosis (Johne's disease), caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map), has not been reported. The latter is also a potential zoonosis, responsible for Crohn's disease in humans (COLLINS et al., 2000; NASER et al., 2000 and 2002; FELLER et al., 2007). The present study describes the simultaneous occurrence of these potential zoonoses and the pathobiology induced by them in goats.

Materials and methods

Clinical symptoms. Five female alpine cross goat carcasses belonging to the Sheep Research Station, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-K were referred to the Division of Veterinary Pathology for necropsy. The farm uses a semi-migratory managerial system, with the flock pasturing in the highlands during the summer season (April to September) and stall -eeding coupled with grazing in lowland pastures during the rest of the year. The goats were 2-2½ years old. The animals were found to be suffering from diarrhoea after returning from highland pastures. Four succumbed to enteric disease and the fifth died of strangulated abdominal hernia. One goat was found to be pregnant.

Necropsy. Concurrent visceral linguatulosis and paratuberculosis were diagnosed in all five carcasses. Morbid pathology was recorded and materials collected for parasitological, cytological, bacteriological and histopathological investigations following standard procedures. Intestinal and lymph node scrapings were collected for confirmation of paratuberculosis by Polymerase Chain Reaction (PCR).

Cytological and bacteriological investigations. Impression smears from the mesenteric lymph nodes and scrapings from various parts of the intestines (duodenum, jejunum, ileum, ileo-caecal junction, caecum, colon and rectum) were made and stained using Wrightes-Giemsa, Grams and Ziehl Neelson's (ZN) staining methods.

Histopathological investigations. Representative tissue pieces from affected organs were fixed in 10% buffered formal saline and processed for paraffin embedding. Tissue sections measuring 4 to 5µm were stained with routine Harris Haematoxylin and Eosin (HE) and Ziehl Neelson's (LUNA, 1968) and Auramine-Rhodamine fluorescence methods (http://www-medlib.med.utah.edu/WebPath/webpath.html). Calcification was demonstrated by Von Khossa's method, counter staining with HE stain.

Parasitological investigations. The parasites (nymphs) collected from mesenteric lymph nodes were washed and maintained in phosphate buffer saline in two groups,

one at room temperature and the other at 4 °C, to check *in-vitro* survivability. The morphometry of 50 parasites was done and the nymphs were examined both by light- and stero-microscopy. Three parasites were processed for scanning electron-microscopy. The parasites were washed in PBS and fixed in 4% gluteraldehyde solution in cacodylate buffer at pH 7.4 for 4 hours at 4 °C, followed by graded dehydration to absolute ethanol, and then dried using liquefied Co_2 at a pressure of 75kg/cm^2 and temperature 32 °C in a critical point dryer, Hitachi model HCP-2. The electrical conductivity of the parasites was enhanced by coating them with gold, using thermal evaporation carried in a vacuum evaporator Hitachi model HUS-5GB at a pressure of 10^{-6} torr. The parasites were examined using a scanning electron microscope Hitachi model S-3000H at various accelerating voltages and magnifications.

Detection of Mycobacterium avium subp. paratuberculosis (Map) by polymerase chain reaction. Extraction of DNA. Pieces of small intestine, revealing corrugations, were cut open longitudinally and washed thoroughly under a stream of sterile, distilled water. The mucosal surface was scraped off, using a sterile scalpel and homogenised in lysis buffer (20 mM Tris HCl; 2 mM EDTA; 1% Triton X). The mesenteric lymph nodes were also homogenised separately in the lysis buffer. The homogenate was centrifuged at 10,000 rpm for 5 minutes and 300 µL of the supernatant used for extraction of DNA using the Guanidine thiocyanate (GTC) Method (CHOMCZYNSKI and SACCHI, 1987).

PCR amplification of IS900. The DNA extracted as described above was used as a template for amplification of a 278 bp fragment from an IS900 sequence, using a set of primers described by SIGURDARDOTTIR et al. (1999), p11 (5/ CGTCGTTAATAACCATGCAG3/) and p36 (5/ GGCCGTCGCTTAGGCTTCGA3/). PCR was performed in a 25 µL reaction mixture containing 2 µL template DNA, 2.5 µL 10 × PCR buffer, 2 mM $MgCl_2$, 2 µL of 100 mM dNTP mixture, 0.2 µM of each primer and 1 U of Taq polymerase in sterile, distilled water. Positive and negative controls were run alongside the samples. Following the initial denaturation at 94 °C for 2 minutes, the reaction mixture was cycled 35 times 94 °C for one minute (denaturation), 68 °C for 1.5 minutes (annealing) and 72 °C for 2 minutes (synthesis) and the final extension at 72 °C for 10 minutes (AHMED et al., 1999). The PCR assay was performed in a Gene Amp PCR System 2400 Thermal Cycler (Applied Biosystems, CA, USA).

*Analysis and detection of products***.** The PCR products were analysed on 1.5% agarose gel containing ethidium bromide (0.5 mg/mL) in Tris-acetate ethylene diamine tetra acetic acid (EDTA) buffer (TAE) (0.04 M Tris-acetate, 0.001 M EDTA) (SAMBROOK and RUSSEL, 2001). The products were visualized with UV illumination and imaged with gel documentation system (GDS 8000 system, UVP, UK).

Results

Clinical signs. Four of the five animals were diarrhoeic with progressive emaciation, anorexia and did not respond to treatment. The animals were recumbent prior to death. One case was reported to have succumbed to traumatic abdominal hernia.

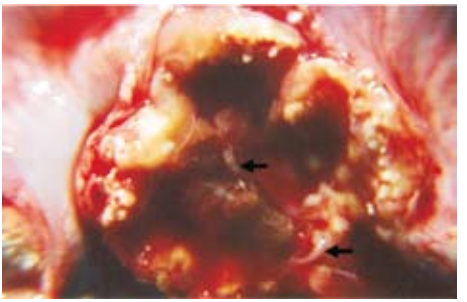

Fig.1. Mesenteric lymph node showing haemorrhages, calcification and presence of *L. serrata* (arrow)

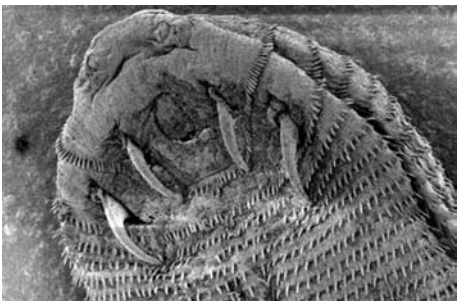

Fig. 2. Scanning electronmicrograph of the anterior-ventral side of the parasite showing a pair of tusk-like clawed hooks on either side of the mouth

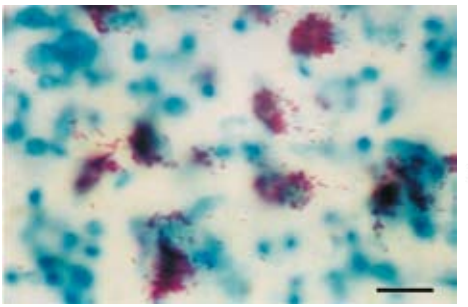

Fig. 3. Impression smear from the mesenteric lymph node revealing clusters of AFB in macrophages. ZN stain, scale bar = 20 µm.

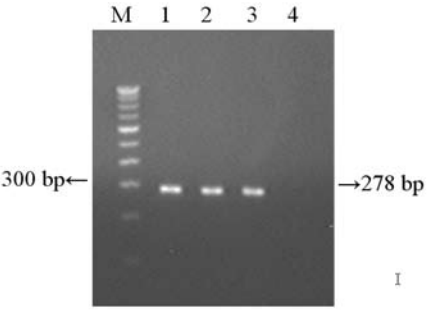

Fig. 4. PCR amplification product of IS900 specific for Map. Lane M 100 bp DNA ladder; Lane 1 Positive Control; Lane 2, 3 Map positive sample; Lane 4 Negative control.

Postmortem observations. The animals appeared weak and emaciated. Gelatinization of subcutaneous and visceral fat was noted. The intestines revealed congestion and thickening of mucosa, especially in the distal small intestine, caecum, proximal colon and proximal rectum. Corrugation was prominent in two cases. The mesenteric lymph nodes (MLN) were enlarged, flabby and oedematous. The serosal surface revealed gelatinization with numerous parasites, nymphs of *L.* serrata. When cut, the lymph nodes exuded fluid; however, in two carcasses they were severely haemorrhagic (Fig. 1). Cystic spaces filled with viscid, turbid fluid, especially towards the cortical area were noted. The parasites were seen both in the subcapsular region as well as in the parenchyma. These were alive and wriggling actively in the parenchyma. Some lymph nodes made a gritty sound when cut. More severe changes were observed in the jejunal and ileal lymph nodes. Additional observations were congested lungs and lightly jaundiced livers.

Parasitological observations. Numerous parasites identified as nymphs of *L. serrata,* were recovered from MLNs. The parasites were milky-white in colour, 4.0-6.0mm long, tongue-shaped, with slightly convex dorsal and flattened ventral surfaces. The body appeared to be divided by transverse rings. The anterior end was broad and thick subterminally and tapered posteriorly. The parasites survived in PBS for 4 days both at room temperature and at -4 °C. On thawing, they showed active motility, especially pushing their anterior ends as if trying to thrust their hooks into the glass. The parasites tried to swim in the fluid media by bending their bodies, along with slight elongation and contraction. On day five, the parasites did not show any movement and were considered dead. Some of the parasites had shed their cuticles. Morphological and morphometrical studies by light-,

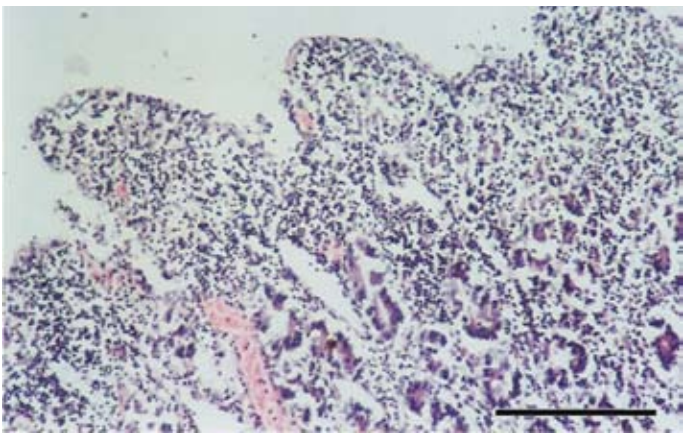

Fig. 5. Section of the ileum showing flattened thumb-like villi heavily infiltrated with mononuclear cells. H&E stain, scale bar = 200 µm.

stero- and scanning electron microscopy revealed a smooth, roughly triangular, anterior region with a caudal base. The anterior, most frontal area was flattened to between 120-130 µm. A pair of lateral prominences with a central depression, giving the appearance of an eye spot, was observed just posterior to it. The width at the posterior margin of the lateral prominences was 250-260 µm, increasing caudally, reaching 400-415 µm at first transverse ring anterior to the ventral mouth and 600-700 µm at approximately the 3rd

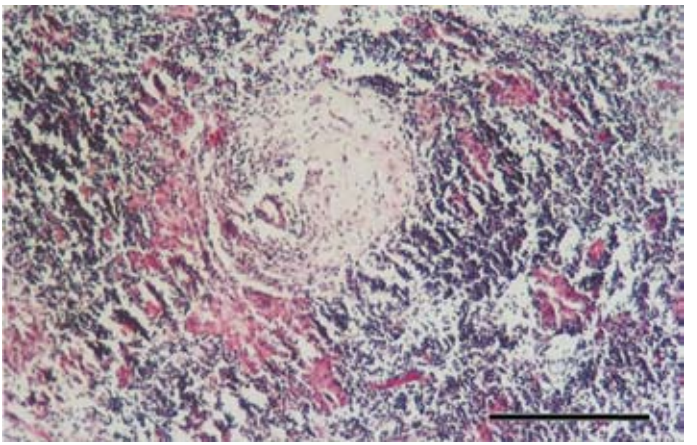

Fig. 6. Section of the ileum showing fibrinoid degeneration and vascuilitis in the submucosal vessels. H&E stain, scale bar = 200 µm.

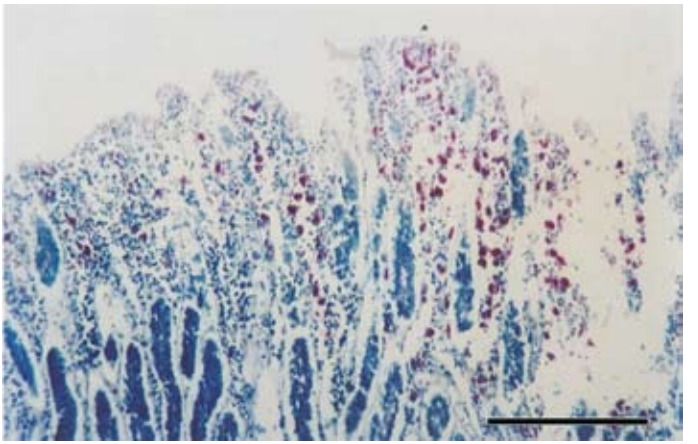

Fig. 7. Section of the ileum showing AFB in the macrophages in the mucosa. ZN stain, scale bar = 200 µm.

transverse ring. Posterior to this, the body tapered gradually, reaching about 130-180 μm at the caudal end. The ventral surface revealed an anterio-posteriorly elongated, oval, sub-terminal oral aperture, about 108-114 μm long and 58-63 μm wide, guarded by a central cushion-like structure. A pair of strong, clawed hooks was observed on either side of the mouth, rising from the hook pit. The hooks had a strong, laterally flattened base, 60-75 μm broad and were 160-175 μm long, curved strongly backwards and inwards after emerging from the pit, resembling walrus tusks. (Fig. 2). Each hook possessed a claw-like structure in the anterior proximal region, arising from within the depression, about 7-9 μm wide at base and 34-36 μm long, with a curved nail like a distal end.The anterior pair of hooks was present at the level of the buccal structure, placed laterally and equidistant from it and about 191-193 μm apart from each other. The second pair of hooks, presenting posterior and lateral to the first pair, was 375-380 μm apart. The anterior and posterior hooks on the epsilateral side were 120-130 μm apart. The cuticle was armed with spines arranged in 82 to 95 transverse rows along the posterior margin of transverse rings. The spines faced towards the posterior and were 5-7 μm at the base and 21-29 μm long, tapering distally, forming single, pointed, bifid or trifid tips. The first row passed just behind the anterior pair of hooks and mouth separating them from second pair of hooks. The first and second rows of spines were 120 to 128 μm apart laterally and 60-63 μm at the mid-ventral region. The interval between the rings decreased towards the posterior region, ranging from 28-34 μm in the anterior, 26-28 μm in middle and 15-18 μm in the caudal region. The distance between the spines in a row was 7-15 μm. The spines had a pyramidal base, which joined their lateral counterparts, giving the appearance of a transverse ridge. Cuticular pores of 1.8×2.3 μm with a cushion were observed, especially along the lateral sides. The gut appeared to extend anterio-posteriorly along the mid-ventral line, marked externally by a longitudinal depression extending from the first to last transverse ring. The dorso-ventrally elongated anal slit, measuring 40-42 μm, was observed at the posterior end.

Cytological observations. Wrights-Giemsa stained impression smears from *L. serrata* infected MLNs revealed the presence of lymphoblasts. Acid-fast bacilli (AFB) were noted both extracellularly as well as intracellularly within macrophages (Fig. 3). These were seen in abundance in mucosal scrapings from the ileo-caecal junction, followed by those of the ileum and jejunum and were still more abundant in the impressions from lymph nodes with areas of calcification. MLNs from the proximal intestinal regions were variably affected and infrequently revealed few AFB. Scraping from the duodenum did not reveal any AFB.

PCR for Mycobacterium avium subsp. paratuberculosis. PCR amplification product of IS900 (278bp) specific for Map was observed in all five cases from intestinal mucosal scrapings and the lymph node (Fig. 4).

Histopathological observations. Histopathologically, the jejunum and ileum were most severely affected, while the duodenum was least affected. Cellular changes were characterised by sparse to diffuse lymphocyte infiltration in the mucosa. Villi in the jejunum and ilium revealed fusion, with broad, flattened thumb-like tips (Fig. 5). Those not fused often showed a club-shaped appearance. Blood vessels appeared to be marginalised, running along the borders of the villi and were congested. Epithelioid macrophages interspersed the diffuse and extensive infiltrative lymphocytes. A few plasma cells were observed. No giant cells were visible. The lamina propria was thickened and distended, compressing and pushing the crypts downwards or obliterating them. Infiltration was predominantly of lymphocytes. Blood vessels were found to contain components of granuloma. The vascular wall revealed fibrinoid degeneration and vasculitis (Fig. 6). Obliteration and thrombosis of the vessels were evident. The submucosa was thickened and oedematous, with dilated lymphatics containing clumps of inflammatory cells. The cellular infiltrate was characterised by an equimixture of lymnphocytes and macrophages. Peyer's patches revealed focal granuloma, characterised by the presence of lymphocytes, epithelioid macrophages and large number of polymorphs. Varying degrees of casseous necrosis and calcification were evident. In places, the entire Peyer's patch was calcified. The serosa was oedematous with dilated lymphatics. Diffuse infiltration of lymphocytes was evident, which was more severe in the perivascular and perilymphatic regions. In general, the epithelioid macrophages showed abundant eosinophilic cytoplasm. Their nuclei were vesicular, with one or two prominent nucleoli and stippled peripheral chromatin. An increased nuclear to cytoplasm ratio was observed. Karyorrhexis was seen, forming multiple small-condensed nuclear remnants within the cells. The mucosa of the ileo-caecal valve revealed diffuse infiltration of lymphocytes, admixed with epithelioid macrophages, which were more concentrated towards the apical region. The lamina propria revealed diffuse infiltration of lymphocytes, fewer epithelioid cells and, rarely, some plasma cells. In the colon and rectum, the mucosa was less severely affected, revealing only sparse infiltration of lymphocytes. However, focal granulomatous lesions consisting primarily of lymphocytes and an equimixture of epithelioid cells and polymorphs were evidenced. These lesions were mainly found associated with blood vessels.

AFB were observed scattered in clusters within the epithelioid macrophages and scattered extracellularly in the mucosa, including the lamina propria, in the jejunum, ilium and ileo-caecal junctions. Macrophages laden with organisms were more numerous along the periphery of the villi (Fig. 7). Only a few AFB could not be demonstrated in the duodenum, colon or rectum. The organisms showed a characteristic reddish-yellow fluorescence following Auramine-Rhodamine staining.

The mesenteric lymph nodes revealed oedema and haemorrhage, with a loss of lymphocytes, giving a moth-eaten appearance. Sections of the parasite,the nymph of

L. serrata, were observed within oedematous cyst-like spaces surrounded by dense lymphocytic zones, often with several lymphoblasts. Also, multifocal epithelioid granulomas were evident. Multifocal caseative necrosis with calcification was observed in some lymph nodes. In severely affected cases, such lesions were coalescent. Scarce to abundant Map was noted within the macrophages as well as extracellularly. Extra-cellular bacilli were more numerous adjacent to the calcified foci.

Other histological changes included congestion, haemorrhaging and emphysema in the lungs, and congestion, hypercellularity and hyper-segmentation in glomeruli, which invariably caused reduction or even obliteration of Bowman's capsule. The uterus of the pregnant doe did not reveal any lesions or AFB.

Discussion

The present investigation records concurrent infections of visceral linguatulosis and paratuberculosis in goats. *L. serrata* is an obligate endoparasite belonging to pentostomids, a unique group of degenerate arthropods, phylogenetically related to branchiuranus. Among the pentostomids, *L. serrata* (*L. rhinosia, L. denticulate*) and *Porocephalus armillatus* (*Armillifer armillatus*) have been found to infect human beings with a noteworthy frequency and are hence recognised as important zoonotics. Carnivorous mammals such as dogs, wolves, foxes and felines act as definitive hosts, with the adult *L. serrata* parasitising nasal airways, frontal sinuses and the tympanic cavity. Herbivorous mammals, including sheep, goats, cattle and rodents act as habitual intermediate hosts. Human beings may act as an intermediate hosts or accidental definitive hosts (PRATHAP, 1981). Nasopharyngeal linguatulosis is diagnosed clinically by laboratory examination of nasal swabs and faeces for eggs. However, visceral linguatulosis poses a diagnostic problem. Visceral linguatulosis warrants diagnosis by biopsy, exploratory laparotomy, ophthalmology, radiological examination or post-mortem examination, supplemented by morphological identification of the nymph. However, so far, diagnosis has been given at post-mortem in herbivores.

The morphological characteristics of the parasites observed were similar to the third-instar larval form of *L. serrata*. Advanced morphological and morphometrical studies of *L. serrata* larvae have been considered important for its differentiation from closely related species e.g. *L. recurvata,* which possess smaller spines (LAZO et al., 1999). In the current study, the observation of a single cuticular ring separating the mouth and first pair of hooks from the second pair of hooks is in accordance with our earlier observation (MIR et al., 2004). The size of the cuticular spines and distance between the rings was found to decrease towards the caudal end, which is in accordance with the observations of LAZO et al. (1999).

Extended *In vitro* survivability of the nymph has also been reported by LAZO et al. (1999), who observed that the parasite continued to be mobile in saline for more than 10 hours. In the present study, not only the period of *In vitro* survivability was found to be prolonged, but the parasites also survived daily refrigeration and thawing. These observations suggest it can survive longer in temperate conditions, in carcasses facilitating the completion of its life cycle.

In animals, the nymph of *L. serrata* has been observed in the mesenteric lymph nodes, liver and lungs (SHEKARFOROUS et al., 2003), while in humans, though less frequently, it has also been reported in the eyes, brain, intestine and prostate gland (LAZO et al., 1999). In the present study, the parasites were observed only in the mesenteric lymph nodes. This is in accordance with the reports that the mesenteric lymph nodes are most often involved in herbivores (ESMAIL et al., 2000). In the present study, paratuberculosis of the multibacillary type was diagnosed in postmortem observations, histopathological lesions and observation of AFB in tissue impression smears and histopathological sections and confirmed by PCR probing for IS 900. Demonstration of AFB in impression smears has been used frequently for diagnosis, but is neither specific nor sensitive (SIVAKUMAR et al., 2004). Since all cases revealed AFB, they might have been in stage III to IV of the disease. However, AFB was not observed in smears from the duodenum or associated mesenteric lymph nodes, which correlated well with the histopathological lesions in the area.

At subspecies level, *Map* can be differentiated from other two members, *M.avium* subsp. *avium* (*M. avium*) and *M. avium* subsp. *silvaticum* (*M. silvaticum*) genotypically, by the presence of multiple copies of an insertion element, IS900. PCR targeting IS900 has been validated and used for detecting Map DNA in peripheral blood, milk, individual and pooled faecal samples, tissues and also human samples. The test has been found to be specific, discriminating among the related species and used in field trials (SOCKETT et al., 1992; WHIPPLE et al., 1992; HARRIS and BARLETTA, 2001).

Gross and histopathological lesions in intestines were characteristic of paratuberculosis and in accordance with earlier reports. Histopathological lesions associated with natural paratuberculosis in ruminants have been categorised on the basis of the presence of granulomatous lesions, the location of granulomas in different gut-associated lymphoid tissue components, the intensity and distribution of lesions, the inflammatory cell types present, and presence and subjective assessment of the number of Map in the lesions (CORPA et al., 2000; GONZALEZ et al., 2005). A spectrum of lesions related to the stage of disease and host immune status have been described. Clinical cases and cases with gross intestinal lesions have been, essentially, associated with two main histopathological forms-the more common, borderline-lepromatous or multibacillary form and less common (30%) paucibacillary form. The former is characterized by extensive, diffuse infiltration

of macrophages containing numerous acid-fast Map, few lymphocytes and granulocytes, and occasional Langhans-type giant cells. The latter is characterized by diffuse infiltration of lymphocytes, with some macrophages and few, if any, AFB. Lesions observed in the present study varied in severity in different parts of the gut and among the animals, suggestive of different grades. The lesions were more severe in the ileal and jejunal regions, corresponding to the multibacillary form, while lesions in the duodenum and rectum were paucibacillary. Animals from a paratuberculosis infected flock can present varying immunological and pathological pictures and be located in different positions in the spectrum, however, the difference in the lesions in different parts of the gut of the same animal correlates well with the primary involvement of the ileal and jejunal regions, usually following an oral infection. Progression of the granulomatous lesions is controlled by finely tuned cytokine production and cell-to-cell interaction, favouring differential recruitment of cells. Thus, lesions at different stages vary in the predominant cell type, which are at different developmental and activation stages. Further, production of TGF-γ, cytokine, down-regulating macrophage activation, has been found to be low in the initial phase of the disease and is up-regulated in macrophages with a higher number of bacteria (PEREZ et al., 2005).

The chromatin condensation and dense nuclear fragmentation of epithelioid macrophages could be attributed to apoptosis and the condensed bodies might represent apoptotic bodies. Antigen specific induced apoptosis has been demonstrated in peripheral blood mononuclear cells from paratuberculosis infected animals, following stimulation with Map PDD (GRELL et al., 2005). Apoptosis has been regarded as an important host defence mechanism against mycobacterial infection, while its inhibition by the bacteria determines the virulence (KEANE et al., 2000)

Blood vascular lesions, like granulomatous arteritis, with thrombus formations, have been described (GONZALEZ et al., 2005) and associated with haematogenous dissemination of Map. The fibrinoid necrosis appears to be due to a type III (immune complex) hypersensitivity reaction. Multibacillary lesions have been associated with high humoral immune response. High level of antibodies in the blood may favour immune complex formation during the haematogenous spread of Map and their precipitation locally in the vessel wall, causing fibrinoid necrosis and thrombosis.

Necrosis and calcification observed in Peyer's patches and mesenteric lymph nodes is not a frequent finding in paratuberculosis and has never been observed in cattle. However, casseous necrosis and tubercle formation associated with Map infection has been observed in sheep, goats and deer (CORPA et al., 2000). Although it is debatable, a possible association with Map infection has been suggested, as its most closely related species, *M. avium,* causes necrosis and calcification. Calcification in lymph nodes may also be attributed to the parasites. It has been suggested that living nymphs provoke little

inflammation, whereas the death of the parasite leads to a prominent host response. In human beings, granulomatous reactions around the encysted parasite and calcification with pseudotubercle formation in the liver have been described (SYMMERS and VALTERIS, 1950). The moth-eaten appearance of MLNs, the presence of lymphoblasts in their sections and impression smears as well as lymphoid hyperplasia around the cystic spaces may be attributed to tissue histeolysis, caused by the feeding habit of the parasite on lymphoid cells and reticuloendothelial cells and the host compensatory response (MEHLHORN, 2001).

In the present investigation, the mesenteric lymph nodes were found simultaneously infected with nymphs of *L. serrata* and Map. This seems to be first report of its kind. However, the presence of the parasite in cattle and humans with tuberculosis has been demonstrated (SYMMERS and VALTERIS, 1950; MURALEEDHARAN and ZAKI, 1975). Further affinity of the parasite for the tuberculous lymph nodes has been suggested (LAPAGE, 1956). The present investigation suggests that the parasite, being lymphovorous, might predispose to the multibacillary form of paratuberculosis.

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SAŽETAK

Istovremena pojava visceralne lingvatuloze i paratuberkuloze dijagnosticirana je u pet križanih alpskih koza (*Capra hircus*). Patomorfološki i patohistološki ustanovljena je multibacilarna paratuberkuloza i promjene uzrokovane parazitima. Paratuberkuloza je dokazana lančanom reakcijom polimerazom upotrebom IS 900. Metode svjetlosne mikroskopije, stereoelektronske mikroskopije i skenirajuće elektronske mikroskopije rabljene su pri determinaciji ličinki trećeg stupnja petoustaša *Linguatula serrata*, Frohlich, 1789. Morfološke promjene pretežito su bile dokazane u crijevima i mezenterijskim limfnim čvorovima. Oštećenja uzrokovana paratuberkulozom bila su uglavnom difuznog multibacilarnoga tipa s jakom granulomatoznom reakcijom koja se očitovala pojavom mnoštva makrofaga, velikim brojem acidorezistentnih štapića, promjenama različite veličine sličnima cistama u limfnim čvorovima te nekrotičnim praznim područjima u parenhimu. Izrazito edematozni i hemoragični limfni čvorovi s kalcificiranim područjima i velikim brojem mikroba *Mycobacterium avium* subsp. *paratuberculosis* (Map) osnovna su značajka istodobne pojave ovih dviju bolesti. Istraživanje upućuje na zaključak da je limfovorni parazit *Linguatula serrata* predisponirajući čimbenik za pojavu multibacilarne paratubekuloze.

Ključne riječi: lingvatuloza, paratuberkuloza, patologija, koze