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Goat paratuberculosis in Chile: first isolation and confirmation of *Mycobacterium avium* subspecies *paratuberculosis* infection in a dairy goat

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Abstract. In October 2004, 41 goats >2 years old from a Saanen dairy goat herd located in Purranque County, 10th Region, Chile, were sampled and tested for paratuberculosis. While collecting samples it was observed that several goats were thin and emaciated. One goat was sufficiently debilitated to warrant humane euthanasia. This animal was brought to the Veterinary School at the Universidad Austral de Chile for necropsy. The goat selected for necropsy was a 12-year-old doe. The animal showed classical clinical signs of caprine paratuberculosis: emaciation despite willingness to eat, dry and rough hair coat, and no evidence of diarrhea. Gross pathology and histopathology of the necropsied goat were consistent with paucibacillary paratuberculosis. Bacteriology, serology, and PCR confirmed the diagnosis. This is the first published report of goat paratuberculosis in Chile confirming a case of caprine paucibacillary paratuberculosis.

Key words: Bacteriology; dairy goats; ELISA; paucibacillary paratuberculosis.

Paratuberculosis (Johne's disease) is a chronic infectious enteric disease that affects domestic and wild ruminants. It is an economically important disease seen primarily in cattle, sheep, and goats and is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Paratuberculosis in small ruminants is widely distributed. The first case in goats was reported in 1912.[19] In the following years the disease was reported in several other European countries, such as Great Britain,[13] Italy,[15] and France,[8] as well as in many Asian and African countries.[3] On the American continent caprine paratuberculosis has been reported in the USA,[9] Argentina,[20] and Canada.[12] Although caprine paratuberculosis is known to be present in Chile (unpublished), it has not been officially reported, nor has the etiological agent been confirmed by biochemical or molecular methods.

In October 2004 goats from a Saanen dairy goat herd located in Purranque County, 10th Region of Chile, were sampled and tested for paratuberculosis as part of a research project evaluating a paratuberculosis diagnostic test in goats. The herd had 75 animals in total, but only all goats >2 years old were sampled (41). During the winter season the herd was confined to a free-stall barn, and the rest of the time it was intensively grazing. Goats were milked mechanically twice daily. The farm kept good records and had no previous history of paratuberculosis, based on clinical evidence or prior laboratory tests. The herd was created 10 years prior to this investigation by purchase of goats from a Saanen goat herd with good genetics and milk production records but with a history of clinical disease compatible with paratuberculosis. However, additions to the herd were made from several other sources with unknown disease history. While collecting samples, several thin and emaciated goats were observed. Herd nutrition or feeding management could not explain these observations. The herd owner had previously observed diarrhea in some of these animals. One goat was sufficiently debilitated to warrant humane euthanasia. This animal, a 12-year-old doe, was brought to the Veterinary School at the Universidad Austral de Chile for necropsy and confirmation of diagnosis. It appeared depressed and according to the owner had become thin over the previous 6 months. The animal was in poor body condition (body condition score 2 out of 5) and had a dry, rough hair coat. The animal ate and drank willingly. Rumination was normal, and feces were normally formed. Clinical pathological analysis performed at the Animal Clinical Pathology Laboratory, Universidad Austral de Chile, showed a low blood concentration of both albumin (20 g/L) and total protein (55 g/L), which is consistent with cachexia.

The necropsy revealed a marked emaciation. The lymphatic vessels on the serosal surface of the jejunum were mildly distended. The duodenum mucosal surface was mildly thickened and corrugated. The jejunum was without gross lesions, and the ileum showed mild thickening. Mesenteric lymph nodes were severely enlarged, and multiple foci of small granulomas with central calcification were detected in the cut surface. The lamina propria of the middle and final portions of the jejunum and ileum were mildly to moderately infiltrated with inflammatory cells, mainly lymphocytes. The same type of mild inflammatory infiltrate was present in the ileal submucosa, and there was a mild thickening of the tips of the villi of the ileum with mild lymphangiectasia (Figs. 1, 2). In the epithelial cells, moderated eosinophilic hyaline material was observed. Lymphocytic inflammatory cells were also found in the caecum and colon. No acid-fast bacteria were seen on any tissue section stained with Ziehl-Nielsen stain (Fig. 3). In the jejunal and ileo-cecal lymph nodes there were many

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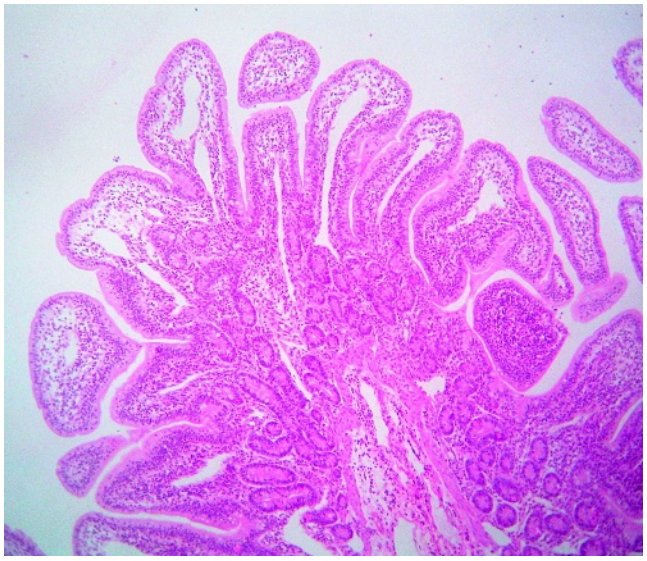

Figure 1. Diffuse inflammatory infiltrate, mainly lymphocytes, present in the lamina propia of the ileum, thickening of the villi tips and dilatation of lymphatic vessels. HE. 10×.

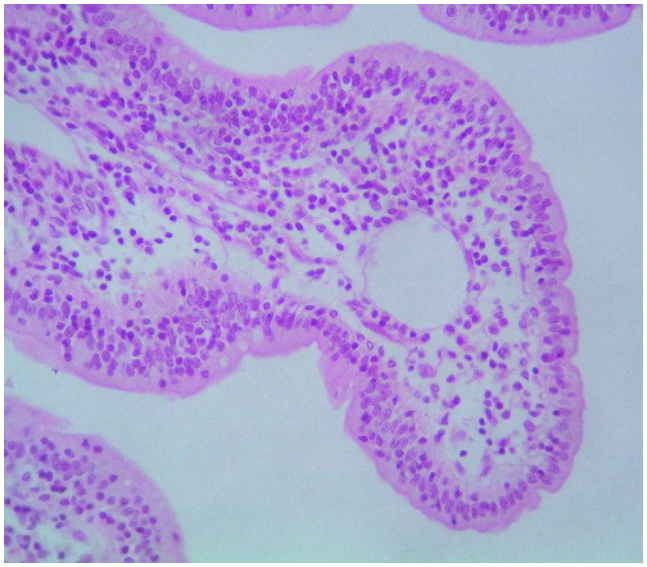

Figure 2. Diffuse inflammatory infiltrate, mainly lymphocytes, in the lamina propia of the ileum with dilatation of lymphatic vessels. HE. 40×.

small to mid-size granulomatous foci, some of them with central calcification. These lesions were located in the perinodal connective tissue, in the outer cortex, and in the diffuse lymphatic tissue of the deep cortex.

Tissues (ileum, duodenum, mesenteric, and ileo-cecal lymph nodes) and feces were processed for isolation of MAP using 3 tubes of Herrold Egg Yolk Medium containing mycobactin J[a] and 1 tube without mycobactin. Sample processing methods followed Cornell University Diagnostic Laboratory recommendations.[18] Suspected MAP colonies (typical colonial morphology and growth rate and showing mycobactin-dependence) were confirmed by IS*900* PCR technology using specific primers for this pathogen (P90 and P91).[17] Growth of the organism was detected after 7 weeks of incubation. Low numbers of MAP were isolated from the ileum (10 cfu/g), ileo-cecal lymph node (5 cfu/g), and mesenteric lymph node (2 cfu/g); no positive culture was obtained from duodenum or feces samples after 9 months of incubation.

Serum and milk samples were collected before necropsy and were tested for antibodies against MAP by ELISA by methods previously described.[16] Briefly, serum samples were tested in duplicate according to the manufacturer.[b] Milk samples were centrifuged and a portion of the skim milk fraction was pipetted from below the cream layer. This milk fraction was then treated like a serum sample with 1 protocol exception: the milk was mixed with ELISA kit diluent at a ratio of 1:2 instead of 1:20, as is done for serum. ELISA optical density (OD) readings at 620 nm were transformed to S/P values as per manufacturer's directions. The recommended S/P cutoff of 0.25 for cattle was used to interpret ELISA results for both serum and milk samples. The serum ELISA S/P value was only 0.75 (positive), but for milk the S/P was only 0.09 (negative). Six out of 41 (14.6%) dairy goats tested in the infected herd were fecal culture-positive, and all MAP cultures were confirmed by PCR IS*900*. Nine (22%) were ELISA-positive using serum, and 5 (12%) were ELISA-positive using milk samples.

Two forms of paratuberculosis have been described: multibacillary and paucibacillary.[4] The multibacillary, or lepromatous, form is characterized by a granulomatous enteritis by macrophages filled with abundant acid-fast bacteria[5]; the paucibacillary, or tuberculoid, form has diffuse lymphocytic infiltrates in the lamina propria, with few or no visible mycobacteria.[5,10,14] The paucibacillary form is seen less often, and may occur more often in sheep than other in animal species.[7] Only 2 other published reports could be found describing paucibacillary paratuberculosis in goats,[2,7] and its prevalence is unknown. However, a study using goats from Spanish goat herds found that 15% to 27% of animals with confirmed paratuberculosis had the paucibacillary type.[7] In Scottish sheep herds, 31% of paratuberculosis cases were found to be paucibacillary.[5] This type of paratuberculosis has been associated with a strong cell-mediated immune response,[1] whereas the multibacillary form has been associated with a strong humoral immune response. This may be one reason for the difficulty in using immunologically based diagnostic tests in sheep.[6] It is not known whether the same holds true for goats, but it is certainly possible, given the many similarities between the 2 species.[2]

The case presented in this study showed classical clinical signs of caprine Paratuberculosis: emaciation despite willingness to eat, dry and rough hair coat, and no evidence of diarrhea. Gross pathology, histopathology, bacteriology, and serology of the necropsied goat were consistent with paucibacillary paratuberculosis.[2] A high number of AFB in the feces and a strong humoral immune response would be expected in a chronically infected animal; however, no MAP was isolated from feces, and the ELISA S/P values were <1.0 in serum and negative in milk. Furthermore,

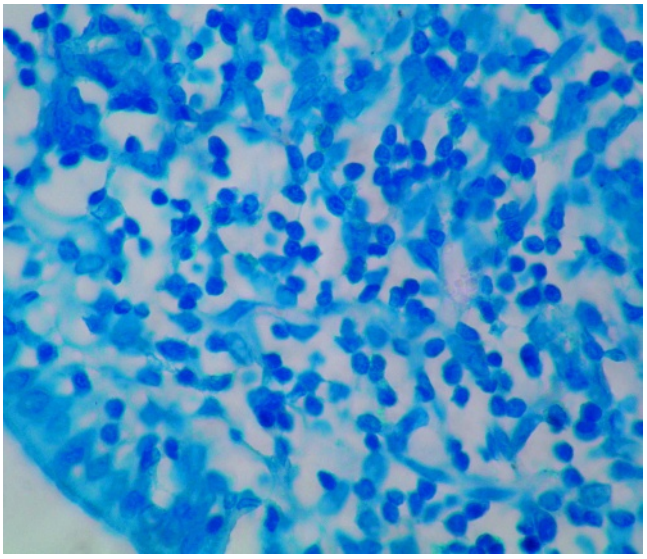

Figure 3. Moderate inflammatory infiltrate, mainly lymphocytes, in the lamina propia of the ileum with no AFB. Ziehl-Nielsen stain. 100×.

although the etiological agent was isolated in low number from the ileum and lymph nodes, no AFB was observed on multiple tissue sections stained with ZN method (Fig. 3). This is the first published report of caprine paratuberculosis in Chile that confirms the less common paucibacillary presentation of paratuberculosis in small ruminants.

The apparent prevalence of MAP infection in the goat's herd of origin was 14.6%, based on fecal culture of all goats >2 years old. Retrospective analysis of herd records indicated that clinical cases of paratuberculosis were a regular occurrence in the herd, suggesting that the infection was introduced at the time the herd was created and continued to spread, consistent with the fairly high infection prevalence. Because most goat farmers are not familiar with Paratuberculosis, it is difficult to recognize it as a clinical problem, and therefore management practices to avoid transmission are not implemented. Transmission of MAP in goats, as well as in other species, occurs mainly by the oral-fecal route. Dissemination of the infection occurs in the latter stages of the disease, resulting in in utero infection of fetuses and excretion of MAP in colostrum and milk.[11] In theory, control of paratuberculosis in dairy goat herds should be similar to that for dairy cattle herds. However, practical implementation of management practices, such as removal of kids from their dams, segregation of young offspring from adults, and exclusive use of milk replacer to feed kids, for many dairy goat herd owners is difficult if not financially impossible. Due to the lack of awareness about paratuberculosis among goat owners, lack of regulations on animal trade, and standard goat husbandry practices, continuing spread of paratuberculosis among and within goat herds should be expected.

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Sources and manufacturers

a Allied Monitor, Inc., Ames, IA.
b IDEXX Laboratories, Inc., Westbrook, ME.

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