

ANATOMOPATHOLOGY OF PARATUBERCULOSIS IN DAIRY  
CATTLE FROM RESENDE - RJ

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## ABSTRACT

Paratuberculosis is an infectious disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) characterized by chronic granulomatous enteritis. The purpose of this work was to report the anatomopathology of three cases of paratuberculosis in autochthonous dairy cattle from Resende, Rio de Janeiro, Brazil. Animals presented characteristic clinical symptoms of paratuberculosis and were seroreactive to ELISA. They were euthanized and necropsied. Small and large intestines, mesenteric lymph nodes and ileocecal valve samples were collected and processed for histopathology and bacteriology. Tissues were fixed in 10% buffered formalin, processed for paraffin inclusion, and stained by HE (haematoxylin-eosin) and ZN (Ziehl-Neelsen). Macroscopic alterations such as small intestine wall

segmental thickness, mucosal hyperaemia, and corrugation were observed. Ileocaecal valve emaciation, evident mesenteric lymphadenomegaly, and lymphangiectasy were also present. The main histopathological findings were enteritis, lymphangitis and granulomatous lymphadenitis. Intestinal lesions were mainly restricted to mucosa and submucosa of jejunum and ileum, characterized by inflammatory infiltration of lymphocytes, eosinophils, epithelioid macrophages, and scarce giant Langhans-type cells. Numerous acid-fast bacilli were observed into macrophages on the top of villi, lamina propria and lymph nodes parenchyma. Anatomopathology was characteristic for the disease and was considered a valuable tool for the diagnosis of paratuberculosis.

KEYWORDS: anatomopathology; bovine; *Map*; paratuberculosis.

ANATOMOPATOLOGIA DA PARATUBERCULOSE EM BOVINOS LEITEIRO DE  
RESENDE- RJ

## RESUMO

A Paratuberculose, uma doença causada pelo *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), é caracterizada por uma enterite crônica granulomatosa. Visamos registrar a anatomopatologia de três casos de paratuberculose em bovinos leiteiros autóctones oriundos de Resende-RJ. Esses animais apresentavam sinais clínicos de paratuberculose e, após teste de ELISA positivo, foram eutanasiados e necropsiados. Amostras de intestino, linfonodos mesentéricos e válvula ileocecal foram colhidas, fixadas em formol neutro tamponado a 10%, processadas e coradas pelo HE (hematoxilina-

eosina) e ZN (Ziehl-Neelsen). Alterações macroscópicas, como espessamento da parede do intestino delgado, mucosas congestionadas e corrugadas, foram observadas. A linfadenomegalia e linfangiectasia também foram evidentes, assim como a congestão e edemaciação das válvulas ileocecais. Os principais achados histopatológicos foram enterite, linfangite e linfadenite granulomatosas. As lesões intestinais se resumiam à mucosa e submucosa, principalmente do jejuno e do íleo e caracterizavam-se por infiltrado inflamatório de linfócitos, eosinófilos e macrófagos epitelióides. A coloração de ZN

demonstrou bacilos álcool ácido resistentes (BAAR) nos macrófagos, no ápice das vilosidades e lâmina própria e, ainda, no parênquima dos linfonodos mesentéricos. A

anatomopatologia foi característica e considerada uma valiosa ferramenta no diagnóstico da paratuberculose.

PALAVRAS-CHAVE: anatomopatológico; bovino; *Map*; paratuberculose

## INTRODUCTION

Paratuberculosis, or Johne's disease, is a chronic, infectious, granulomatous enteritis, which affects domestic and wild ruminants (CHIODINI et al., 1984) and whose etiologic agent is *Mycobacterium avium* subsp. *paratuberculosis* (*Map*). Paratuberculosis causes progressive emaciation, thickening and corrugation of the small intestine's wall, particularly the last part of the small intestine, the ileum, besides mesenteric lymph node enlargement (RIEMANN et al., 1979; COLLINS, 1999). The histopathologic substrate of such inflammatory process is constituted of an infiltrate of epithelioid cells and of scarce Langhan's giant cells present in the intestinal mucosa and submucosa, which, however, do not reveal signs of necrosis or ulcerations (JONES et al., 1996; CLARKE, 1997). Animals get infected with *Map* by contact with other infected animals' manure, by drinking milk or colostrum from an infected animal, or even from a contaminated intrauterine environment (SWENEY, 1996).

Bovines that develop this disease are, in general, those infected in their first weeks of life. The disease manifests itself in animals older than two years of age (SWENEY, 1996). Stressful situations as calf delivery and nutritional deficiency may cause the appearance of the first clinical signs of the disease (KREEGER, 1991). According to BLOOD et al. (1989), the disease is rampant in confined animals, since the environment is greatly contaminated with feces.

Paratuberculosis is responsible for great economic losses in cattle breeding, due to productivity reduction, greater susceptibility of the animals to other diseases, rising of sanitary cost, and greater early discard rate. It is considered nowadays one of the most serious and costly diseases to affect dairy, beef, and, specially, pure animals (COLLINS, 1994; HUTCHINSON, 1996).

The most commonly used tests in the diagnosis of Paratuberculosis can be divided in two main categories: methods to detect the causative bacterium, such as bacterial culture, molecular methods with genetic probes or PCR, and histological examination of target-tissues confirming the presence of alcohol-acid resistant bacillus (BAAR); and methods to detect antibodies

in serum, being ELISA the most used one. (BUERGELT & GINN, 2000). The isolation of the agent from feces or tissues is the gold standard diagnostic method; however, due to a lack of qualification of veterinary bacteriology laboratories on mycobacteriology and to difficulties inherent to *Map*'s cultivation, it is infrequently performed in Brazil (RISTOW et al., 2007).

The main macroscopic lesions appear in the small intestine characterized by the thickening of the mucosa, which presents a typical corrugation of mucosa, associated with mesenteric lymph node enlargement (GONZÁLEZ et al., 2005). The most relevant histopathological findings consist of granulomatous enteritis, lymphangitis and lymphadenitis, associated with BAAR inside macrophages (CHIODINI et al., 1984; KREEGER, 1991).

A differential diagnosis of Paratuberculosis and other chronic processes which lead to progressive weight loss (cachexia), such as malnutrition, tuberculosis, iron and cobalt deficiency, molybdenum intoxication, parasitosis, and chronic liver diseases is necessary (BLOOD et al., 1989).

The purpose of this work was to report the anatomopathology of three cases of paratuberculosis in diagnosed bovines from Resende, Rio de Janeiro, Brazil.

## MATERIAL AND METHODS

Three bovines from two dairy farms (A and B), located in Resende, Rio de Janeiro, Brazil, with were euthanized and necropsied. Farm (A), which produces type B milk through a semi-intensive system, had a herd of approximately 340 half-breed Girolando cattle, where the systems of stud farm and artificial insemination with semen imported from Argentina and Uruguay are used. Farm (B) also produces milk, only that with a semi-intensive system for the production of type C milk. Its herd summed up approximately 150 half-breed Girolando cattle. A significant number of cattle were bought from farm A.

The animals showed clinical symptoms of the disease such as chronic diarrhea and cachexia and were reactive to the serum ELISA test. Serum

was tested with a previously standardized indirect ELISA (MARASSI et al., 2005). Briefly, the test used paratuberculosis protoplasmatic antigen (PPA Allied Monitor), with serum pre-absorbed with *M. phlei*, and an anti-bovine IgG conjugate (Sigma-Aldrich Laboratories). Optical density (OD) was measured at a wavelength of 405 nm, and a cut-off point of 0.350 was chosen as the value giving the most accurate division of positive and negative sera. The animals that presented characteristic clinical symptoms of paratuberculosis and were seroreactive to ELISA were euthanized and necropsied.

Samples of small intestine (duodenum, jejunum, and ileum) and large intestine (colon, cecum, and rectum), mesenteric lymph nodes, and ileocecal valve were collected and fixed in 10% neutral buffered formalin, processed in the automatic tissue processor Leica TP-1020 for the inclusion of paraffin. Sections of 5  $\mu\text{m}$  were cut and stained by the routine method, haematoxylin-eosin (HE) and special, Ziehl-Neelsen (ZN).

## RESULTS

Two animals (animals 1 and 2) out of the three animals with suspicion of paratuberculosis belonged to the same farm (farm A). According to the farm owner, decreases in the production of milk along with chronic diarrhea episodes in adult animals have been observed, at least over the six years, followed by death due to diarrheic frame and progressive weight loss.

Animals 1 and 2 were approximately five-and-six year old Girolando females, which had been suffering from chronic diarrhea, progressive weight loss, and emaciation for six months. Appetite and reproductive functions were normal, and, despite vermifuged and treated with antibiotics, the animals were cachectic, with bristled and dull fur, and showed no clinical improvement.

Animal 3 belonged to farm B and was a two-year-old half-breed male. Despite normal appetite, it had been giving away, for a month, evident signs of cachexia, apathy, liquid, green and mucus diarrhea. The farm owner stated the death of 12 animals with chronic diarrhea and cachexia from 2001 through 2002. The main clinical suspicion was of paratuberculosis.

The three animals, in awful nutritional state, were necropsied. It was possible to examine thickened intestine walls in all three of them, specifically the small intestine. In animal 3, thickening occurred in both small and large intestines. There were as well adherence between

intestinal loops, hyperemia, mucus and severe corrugation on mucosal surface in various segments of the intestine (Figure 1). Lymphadenomegally and lymphangiectasia were evident in all three cases, as well as congestion and emaciation in the ileocecal valves.



FIGURE 1. Segment of bovine small intestine. Paratuberculosis. Intestine with thickening, corrugation and mucosa hyperemia.

The main findings were observed in the small intestine, in the mesenteric lymph vessels and lymph nodes, representing granulomatous enteritis, lymphangitis and lymphadenitis. Intestinal lesions were restricted to the mucosa and submucosa, mainly in the jejunum and in the ileum and were characterized by lymphocytes, eosinophils, and epithelioid macrophages infiltrates (Figura2). Granulomatous reaction was evident mostly on the top of villi and lamina propria (Figura 3)

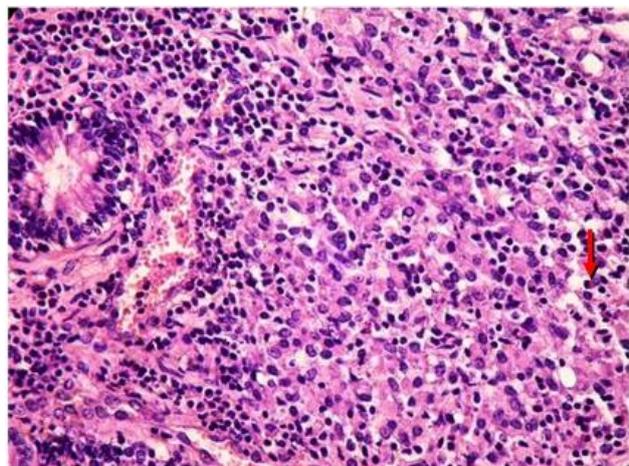


FIGURE 2. Ileal submucosa of bovine. Paratuberculosis. Presence of lymphocytes, eosinophils (arrow), and epithelioid macrophages infiltrates. HE. 400X.

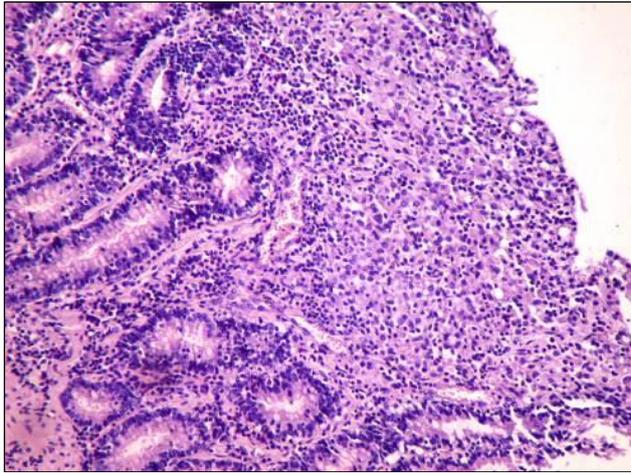


FIGURE 3. Ileal mucosa of bovine. Paratuberculosis. Granulomatous reaction evident on the top of villi and lamina propria. HE. 100X.

By special stain ZN, acid fast bacilli (AFB) were observed in macrophages, most of the times in the form of cluster on the intestinal mucosa, especially on the top of villi and lamina propria (Figure 4) and still on the mesenteric lymph nodes parenchyma

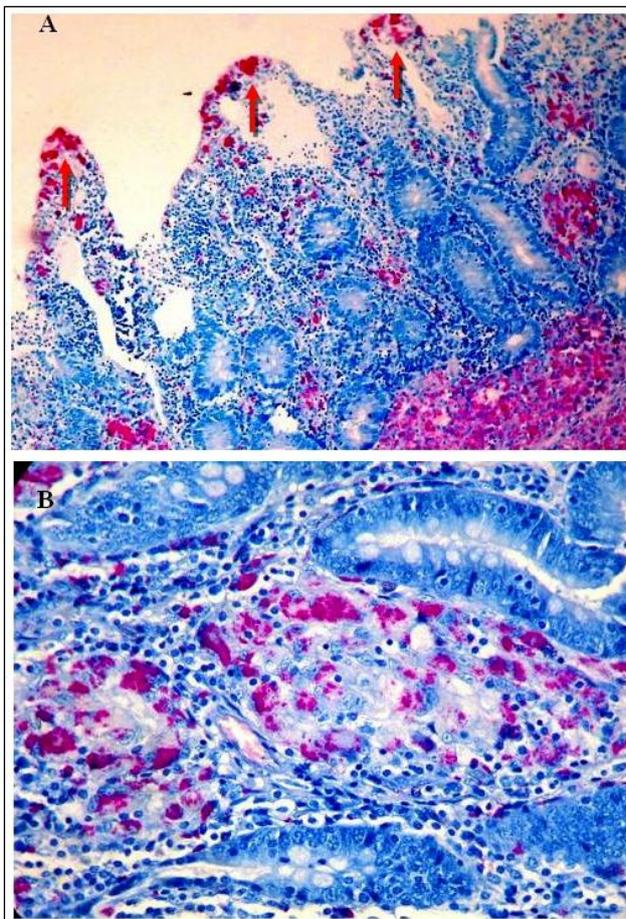


FIGURE 4. Bovine intestinal mucosa. Paratuberculosis. Small intestine mucosa revealing infiltrate of epithelioid cells and presence of multifocal lesions with AFB in the apex of the villosities (arrows) (A), ZN, 100X and lamina propria (B). ZN, 400X.

At microscopy of mesenteric lymph nodes revealed lymphangitis with granulomatous inflammation on cortical and medullar with cell infiltrate with numerous epithelioid macrophages (Figure 5).

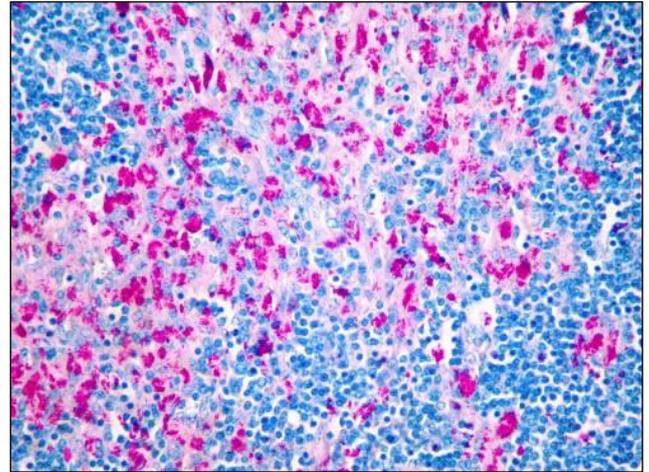


Figure 5. Lymph node of bovine. Paratuberculosis. granulomatous reaction on cortical revealing infiltrate of epithelioid cells and cluster of acid fast bacilli. ZN.400X.

## DISCUSSION

Clinical suspicion of paratuberculosis in the three animals was confirmed based on the clinical signs, positive ELISA tests, on necropsies and histopathological findings and through gold diagnosis, that is isolation of the etiologic agent, conventionally confirmed by mycobactin J dependency tests. Clinical signs as well as macroscopic and microscopic alterations in the three animals were similar to the ones described in cases of bovine paratuberculosis (JONES et al., 1996; CLARKE, 1997; COLLINS, 1999). The chronic diarrhea episodes, intermittent at times, were also evidenced in this study, according to BLOOD et al. (1989), WHITLOCK and BUERGELT (1996).

In the described cases of paratuberculosis, the presence of AFB in the macrophages and epithelioid cells are common findings, whereas in these investigation samples, a great quantity of AFB could be observed in the lesions of the intestinal mucosas and submucosas, which clustered in bacilli, similar to the ones observed by CARRIGAN and SEAMAN, 1990; CORPA et al., 2000, RISTOW et al., 2008.

The paratuberculosis characteristic clinical signs, based on macroscopic and microscopic changes, which were present in the animals studied, were in accordance with the ones that have already been described in other bovines infected by

*Mycobacterium avium* subsp. *paratuberculosis* (CHIODINI et al., 1984; GONZÁLEZ et al., 2005). The paratuberculosis case diagnosis in this study was based on epidemic data assessment of the clinical signs, the necropsy and the microscopic findings. The lesion localization and the granuloma constitution, associated with the lesion type, were similar to the findings by RAMOS et al. (1994); DREIMEIER et al. (1999); HUDA & JENSEN, (2003).

### CONCLUSIONS

The thickening of enteric wall, lymph nodes enlargement and the corrugated aspect of the enteric mucosa were observed through necropsy. Histopathological findings of the epithelioid granuloma with scarcely differentiating Giant cells and presence of AFB have also been identified, mainly on ileal mucosa and submucosa. The clinical sign of chronic diarrhea is not specific for paratuberculosis, and is observed in several other diseases. Nevertheless, it is necessary to perform a differential diagnosis with other chronic processes which lead to cachexia. The anatomopathology, especially the ZN stain, revealed pathognomonic findings for paratuberculosis.

Paratuberculosis granuloma in bovine does not present caseification, mineralization and it shows scarce gigantocitary answer, different from that of the tuberculosis in this specie.

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