CORRELATION BETWEEN PATHOLOGICAL FINDINGS AND BACTERIOLOGICAL CULTURE ON PARATUBERCULOUS CATTLE

Paula Ristow,[1] Ana Bárbara Freitas Rodrigues,[2] Leila Souza Fonseca,[3] Walter Martin Roland Oelemann,[4],Guilherme Nunes Souza,[5] Carla Dray Marassi,[6]
Eulógio Carlos Queiróz de Carvalho[7] and Walter Lilenbaum [8]

1. Pesquisadora visitante do Centro de Pesquisas Gonçalo Moniz- FIOCRUZ
2. Professora do Departamento de Anatomia – Universidade Estadual do Norte Fluminense
3. Professora titular – Universidade Federal do Rio de Janeiro
4. Professor adjunto de Imunologia – Universidade Federal do Rio de Janeiro
5. Pesquisador EMBRAPA
6. Doutora em microbiologia – Universidade Federal Fluminense
7. Professor Associado I do Setor de Anatomia Patológica do Hospital Veterinário/UENF
8. Professor associado em Bacteriologia do Departamento de Microbiologia e Parasitologia da Universidade Federal Fluminense

ABSTRACT

Paratuberculosis (PTB) is a chronic enteritis determined by *Mycobacterium avium* subsp. *paratuberculosis* (Map) that affects ruminants. Three crossbred dairy cattle with clinical PTB were necropsied; tissue samples were collected and processed for histopathology and culture. Intestinal lesions were characterized in three levels of severity (mild, moderate and severe) by gross examination. Gross examination, microscopy and tissue culture were analyzed and compared. Mean tissue culture sensitivity was 66.6%. Map was recovered from 42.8% of intestinal mild lesions, 55.5% of intestinal moderate lesions, and 100% of severe intestine lesions. The results indicate a significant correlation between the level of lesions severity and the isolation of Map.

KEY WORDS**:** Cattle, Johne's disease, Map, paratuberculosis, pathology.

RESUMO

CORRELAÇÃO ENTRE ACHADOS HISTOPATOLÓGICOS E CULTURA BACTERIOLÓGICA EM GADO COM PARATUBERCULOSE

A paratuberculose (PTB) é uma enterite crônica determinada por *Mycobacterium avium* subsp. *paratuberculosis* (Map) que afeta ruminantes. Necropsiaram-se três animais mestiços com PTB clínica, para coleta de amostras de tecidos coletadas, as quais foram processadas para histopatologia e para cultura bacteriológica. Classificaram-se as lesões intestinais em três níveis de severidade (leve, moderada e severa) por exame macroscópico. Procedeu-se ao exame visual, à microscopia e à cultura dos tecidos, comparando-se os resultados entre si. A sensibilidade média da cultura foi de 66,6%. Isolou-se Map a partir de 42,8% das lesões intestinais leves; 55,5% das lesões moderadas e 100% das lesões severas. Os resultados indicam uma correlação significativa entre a variação de severidade das lesões e o isolamento de Map.

PALAVRAS-CHAVES: Bovinos, doença de Johne, Map, paratuberculose, patologia.

INTRODUCTION

Paratuberculosis (PTB) or Johne's disease is a chronic granulomatous enteritis of ruminants characterized by untreatable diarrhoea accompanied by progressive weight loss caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map). The anatomopathological examination of lesions is a valuable definitive diagnostic tool, which allows faster confirmation of clinical cases after the observation of characteristic findings, i.e. thickening of intestines, mucosal corrugation, enlargement of mesenteric lymph nodes, and dilatation of lymphatics (GONZÁLEZ et al., 2005).

The isolation of the agent from feces or tissues is the gold standard diagnostic method; but 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. Map's culture is very slow, takes 8-16 weeks of incubation and is mycobactin dependent (RISTOW et al., 2007). Therefore, the knowledge about the best tissue samples to be collected and processed for bacteriology is mandatory for the diagnostic success.

Although lesion classifications have been made in sheep (PÉREZ et al., 1996) and goats (CORPA et al., 2000), few studies were directed towards the histological classification of lesions of bovines with clinical PTB (BUERGELT et al., 1978; GONZALEZ et al., 2005). The purpose of the present study was to demonstrate the correlation between pathological findings at necropsy and bacteriological culture on paratuberculous cattle, in order to help the collecting of samples and therefore maximize the possibility of recovering the agent from tissues.

MATERIAL AND METHODS

Three crossbred dairy cattle (H, J and G) from two infected herds in Rio de Janeiro State, Brazil, were studied. Paratuberculosis had been diagnosed previously in those herds by serological screening and was confirmed by fecal culture of seroreactive animals (RISTOW et al., 2007). The investigated animals presented characteristic symptoms of PTB and were in poor condition due to chronic diarrhea. After bacteriological confirmation, animals were sacrificed and submitted to a complete necropsy. Small intestine samples (n=24) with varying degrees of tissue damage were collected. Half of each tissue sample was refrigerated for bacteriology and the other half was fixed in 10% neutral buffered formalin.

Samples were processed for histopathology and bacteriology examination. For histopathology fixed tissue samples were embedded in paraffin wax, sections of 5 micrometers were cut and stained by hematoxylin-eosin (HE) and Ziehl-Neelsen (ZN). Tissue culture was performed according to OIE standards (WORLD ORGANIZATION FOR ANIMAL HEALTH, 2005) and inoculated in Herrold's egg yolk (HEY) media containing antibiotics and supplemented either with or without mycobactin J (Allied Monitor). All isolates were confirmed as Map by ZN smears and IS900 PCR, as previously described (RISTOW et al., 2007).

Based on the classification proposed by BUERGELT et al. (1978), intestinal samples were classified in three levels, according to the severity of the lesions, as mild, moderate and severe. The parameters used for the characterization were based on thickness of intestinal wall, mucosal hyperemia, presence of mucus on mucosal surface, grades of corrugation, thickness of mucosa and submucosa; presence of granulomatous lesions, location of granulomas, different cell types observed in the inflammatory infiltrate, and presence of acid-fast bacilli (AFB). Intestinal wall thickness was measured in centimeters with a pachymeter and thicknesses of mucosa and submucosa were independently measured using morphometric analysis (Digital Analysis Morphometric Program); for each sample one field was measured three times and the average was calculated.

Lesions were classified as mild when gross analysis presented intestinal wall slightly thickened, mucosa with focal hyperemia, absence of corrugation, and few or absence of mucus on mucosa surface. Moderate lesions presented moderate thickness of intestinal wall, focal hyperemia,

moderate corrugation of mucosa, and presence of serous mucus or purulent mucus. Severe lesions were characterized by exuberant lesion, with intense thickness of intestinal wall, highly corrugated mucosa, diffuse hyperemia, and presence of serous or purulent mucus.

Statistical analysis was conducted using generalized linear model (MORGAN et al., 2001), Mann & Whitney test and Spearman´s correlation (SAMPAIO, 2002) in order to compare the variables severity of the lesions (low, moderate or severe) in relation to results of the bacteriological culture. Results of the statistical tests were interpreted altogether.

RESULTS AND DISCUSSION

The three animals presented thickness of intestinal wall, typical corrugation of mucosa, lymph node enlargement, and lymphangiectasis. Macroscopic lesions were segmental and varied in severity. Enteritis was restricted to mucosa and submucosa, characterized by inflammatory cell infiltrate with lymphocytes, eosinophils, epithelioid macrophages, scarce Langhans giant cells, and AFB in the top of the villi and the lamina propria.

Mild lesions presented wall thickness of 0.4 - 0.7 cm, mucosa thickness of 696.24 - 727.60 µm, and submucosa thickness of 55.29 - 782.91 µm. Microscopic analysis of those lesions showed multifocal multibacillary lesion, with discrete to moderate infiltrate of epithelioid cells, eosinophils, lymphocytes, and differentiating giant cells. For moderate lesions, observed measures were: wall thickness (0.5 - 0.8 cm), mucosa thickness (764.14 - 1612.29 µm), and submucosa thickness (217.4 - 1071.46 µm). Microscopically, those lesions were multifocal multibacillary, with moderate infiltrate of epithelioid cells, eosinophils and lymphocytes, with differentiating giant cells. The observed measures for severe lesions were: wall thickness (0.8 - 1.8 cm), mucosa thickness (877.36 - 1397.4 µm), and submucosa thickness (140.74 to 1165.14 µm). Microscopic analysis characterized those lesions as being multifocal to diffuse multibacillary, with intense infiltrate of epithelioid cells, eosinophils and lymphocytes, with differentiating giant cells. Animals H and J presented the three levels of lesion in distinct sections of small intestines, while animal G presented only mild and severe lesions, as shown in Table 1.

TABLE 1. Bacteriological findings and severity of small intestinal lesions in paratuberculous cattle.

Sample number	Animal	Lesion level	Culture result
1	H	Mild	Negative
2	H	Mild	Negative
3	H	Mild	Negative
4	H	Moderate	Negative
5	H	Moderate	Negative
6	H	Moderate	Negative
7	H	Severe	Positive
8	H	Severe	Positive
9	H	Severe	Positive
10	H	Severe	Positive
11	J	Mild	Negative
12	J	Mild	Positive
13	J	Moderate	Negative
14	J	Moderate	Positive
15	J	Moderate	Positive
16	J	Moderate	Positive
17	J	Moderate	Positive
18	J	Moderate	Positive
19	J	Severe	Positive
20	J	Severe	Positive
21	G	Mild	Positive
22	G	Mild	Positive
23	G	Severe	Positive
24	G	Severe	Positive

Sixteen (66.6%) of the 24 samples cultured yielded Map. The correlation between the three levels of intestinal lesions and culture results is depicted in Table 1. Map was recovered from three of the seven (42.8%) samples with mild lesions, from five of the nine (55.5%) samples with moderate lesions, and from all eight (100%) samples with severe lesions.

The generalized linear model demonstrated that variables animal and severity of lesions were significant on bacteriological culture results, with an adjusted determination coefficient of 0.64. Mann & Whitney test indicate that the degree of lesion was highly significant to recovery of Map

at the bacteriological culture. Spearman´s coefficient also showed a positive and significant correlation between these two variables. Therefore, results of statistical analysis, when interpreted altogether, strongly suggest that the lesions degree influenced the results of the bacteriological culture.

Our findings demonstrate lesions with cell infiltration restricted to mucosa and submucosa of small intestines. BUERGELT et al. (1978) also described advanced lesions in the small intestines, with macrophage and giant cells infiltration in tunica muscularis and serosa. Giant cells were present in all samples. Those cells represent frequently the only cell type in minimal PTB lesions (BUERGELT et al., 1978) and have been identified in all types of lesions, making this a characteristic of PTB in cattle (GONZÁLEZ et al., 2005).

Macroscopic lesions were segmental and varied in the degree of severity. Bacterial culture of intestine tissues is of particular diagnostic value (HUDA & JENSEN, 2003), but due to the fastidious growth of Map and the variability of the distribution of lesions, insufficient sampling may be a problem (GONZÁLEZ et al., 2005). Measurement and gross examination was useful for clearly distinguishing three categories of lesions, and recovery of Map varied significantly according to the severity of lesions. This finding agrees with GONZÁLEZ et al. (2005), who reported 100% positive culture results from diffuse lesions, 55.5% from multifocal lesions and 37% from focal lesions. Those rates are very close to our findings and clearly demonstrate that the adequate collection of tissue samples, with priority for intestinal sections with typical PTB lesions, may significantly increase the possibility of Map recovering.

In the present study, overall culture sensitivity was 66.6%, but we observed an increase in culture sensitivity of up to 100% in correlation with the increase of lesion severity. Additionally, from one animal (H) Map was only recovered from severe lesions. Although the three lesions categories were multibacillary, there were possibly differences in the number of bacilli, which could have increased culture sensitivity in the severe lesions. Therefore, we recommend that severe exuberant lesions gain priority during collection of samples for culture and histopathology in the diagnosis of paratuberculosis. We believe this study will help in the recognition of PTB lesions, as well as in the collection of samples for its diagnosis.

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REFERENCES

BUERGELT, C.D.; HALL, C.; MCENTEE, K.; DUNCAN, J.R. Pathological evaluation of paratuberculosis in naturally infected cattle. **Veterinary Pathology**, v.15, p. 196-207, 1978.

CORPA, J.M.; GARRIDO, J.; GARCÍA-MARÍN, J.F.; PÉREZ, V. Classification of lesions observed in natural cases of paratuberculosis in goats. **Journal of Comparative Pathology**, v.122, p.255-265, 2000.

GONZÁLEZ, J.; GEIJO, M.V.; GARCIA-PARIENTE, C.; VERNA, A.; COERPA, J.M.; REYES, L.E.; FERRERAS, M.C.; JUSTE, R.A.; GARCÍA MARÍN, J.F.; PÉREZ, V. Histopathological classification of lesions associated with natural paratuberculosis infection in cattle. **Journal of Comparative Pathology**, v.133, p.184-196, 2005.

HUDA, A.; JENSEN, H.E. Comparison of histopathology, cultivation of tissues and rectal contents, and interferon-gamma and serum antibody responses for the diagnosis of bovine paratuberculosis. **Journal of Comparative Pathology**, v. 129, p. 259-267, 2003.

MORGAN, G. A.; GRIEGO, O. V.; GLOECKNER, G.W. **SPSS for Windows**: an introduction to use and interpretation in research. New Jersey: Lawrence Erlbaum Associates, Inc., Publishers, 2001. 214 p.

PÉREZ, V.; GARCÍA MARÍN, J.F.; BADIOLA, J.J. Description and classification of different types of lesion associated with natural paratuberculosis infection in sheep. **Journal of Comparative Pathology**, v.113, 107-122, 1996.

RISTOW, P.; MARASSI, C.D.; RODRIGUES, A.B.F.; OELEMANN, W.M.R.; ROCHA, F.; SANTOS, A.S.O.; CARVALHO, E.C.Q.; CARVALHO, C.B.; FERREIRA, R.; FONSECA, L.S.; LILENBAUM, W. Diagnosis of paratuberculosis in a dairy herd native to Brazil. **The Veterinary Journal**, v. 174, p. 432-434, 2007.

SAMPAIO, I.B.M. **Estatística aplicada à experimentação animal**. Belo Horizonte: Fundação de Ensino e Pesquisa em Medicina Veterinária e Zootecnia, 2002. 265 p.

WHIPPLE, D.L.; CALLIHAN, D.R.; JARNAGIN, J.L. Cultivation of *Mycobacterium paratuberculosis* from bovine fecal specimens and a suggested standardized procedure. **Journal of Veterinary Diagnostic Investigation**, v. 3, p. 368-373, 1991.

WORLD ORGANIZATION FOR ANIMAL HEALTH. **Manual of standards diagnostic tests and vaccines.** Part 2, section 2.2, chapter 2.2.6. Available from: http://www.oie.int/eng/normes/mmanual/A_00045.htm. Accessed: 18 September 2005.

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