Trop Anim Health Prod (2016) 48:1497–1501
DOI 10.1007/s11250-016-1100-z

SHORT COMMUNICATIONS

Paratuberculosis in buffaloes in Northeast Brazil

Marilene de Farias Brito[1] · Alessandra dos Santos Belo-Reis[2] · José Diomedes Barbosa[2] · Daniel Guimarães Ubiali[1] · Ana Paula de Castro Pires[1] · Elizabeth Sampaio de Medeiros[3] · Renata Pimentel Bandeira de Melo[3] · Pedro Paulo Feitosa de Albuquerque[3] · Elise Yamasaki[3] · Rinaldo Aparecido Mota[3]

Received: 12 February 2016 / Accepted: 13 June 2016 / Published online: 22 June 2016

Abstract Several farms in the Northeast of Brazil were investigated for *Mycobacterium avium* subsp. *paratuberculosis* infection in order to identify the occurrence of paratuberculosis in buffaloes. Samples were obtained from 17 farms, two slaughter houses, and a quarantine area in the Northeast. About 15,000 buffaloes of the Murrah, Mediterranean, and Jafarabadi breed as well as their crossbreeds were evaluated for meat, dairy, and mixed farms with semi-intensive or extensive breeding practices. For diagnostic purposes, postmortem and histopathological examination, including Ziehl-Neelsen test of fecal smears and scraped intestinal mucosa were performed. PCR was applied for fecal samples, mesenteric lymph nodes, and intestines. Six Johne's disease-positive farms, which together with those previously identified, indicate that the disease is spread through the Brazilian Northeast, similar to what occurs in cattle herds in other regions of the country. The increase in prevalence of paratuberculosis is a consequence of introduction of animals from other regions without adequate veterinary assistance and due to the little official attention paid to this initially silent and chronic disease.

✉ Marilene de Farias Brito
marilene@ufrrj.br

[1] Setor de Anatomia Patológica, Departamento de Epidemiologia e Saúde Pública, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Rodovia BR465 Km7, Seropédica, RJ 23891-000, Brazil

[2] Centro de Diagnóstico Veterinário, Escola de Medicina Veterinária, Universidade Federal do Pará (UFPA), Campus Castanhal, Rua Maximino Porpino da Silva 1000, Centro, Castanhal, PA 68740-080, Brazil

[3] Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco (UFRPE), Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE 51171-900, Brazil

Keywords *Mycobacterium avium* subsp. *paratuberculosis* · Johne's disease · Ruminants · Water buffalo · Pathology · Molecular diagnostics

Introduction

The occurrence of paratuberculosis (Johne's disease) was first documented in Brazil in the State of Rio de Janeiro by Dupont in 1915. *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) is the etiologic agent which produces chronic granulomatous enteritis in ruminants. Paratuberculosis has been recorded from 12 Brazilian states in buffalo, cattle, sheep, and goats (Yamasaki et al. 2013b). Strategies for control of the disease in the country are insufficient, and economic losses are underestimated (Brito et al. 2014). The disease has formally been reported in buffaloes in Pernambuco (Mota et al. 2010), Maranhão (Barbosa et al. 2010), and Rio Grande do Sul (Dalto et al. 2012). It is crucial to intensify epidemiological studies in the northern and northeastern regions of the country where the number of buffaloes is significant. The objective of this paper is to report farms positive for paratuberculosis in the Northeast Brazil.

Material and methods

About 15,000 beef and dairy Murrah Mediterranean and Jafarabadi buffaloes and their crossbreeds were examined. Extensively and poorly managed herds were investigated as well as those raised under semi-intensive conditions, with reasonable management but excellent animal performance. Seventeen beef or dairy farms in Maranhão, Paraíba, Alagoas, and Bahia, and two slaughterhouses in Pernambuco were visited in order to identify cases of paratuberculosis. Samples from a quarantine

area in Ceará were also obtained for histopathological exam. Necropsies were performed on 26 buffaloes that were euthanized according to the euthanasia guidelines recommended by Normative Resolution No. 13 of September 20, 2013 from the National Council for the Control of Animal Experimentation (CONCEA). The samples obtained were fixed in 10 % formalin, routinely processed, and stained with hematoxylin and eosin (H&E); lesions suggestive of paratuberculosis were Ziehl-Neelsen (ZN) stained. Swaps of ileal mucosa scrapings, fecal smears, and mesenteric lymph node imprints were performed, fixed in methanol, and ZN stained (Bartlett 2013).

Fecal samples and fragments of mesenteric lymph nodes, ileum and cecum, removed with a sterile scalpel, were placed into Eppendorf polyethylene tubes and stored at −20 °C for processing. DNA extraction was performed on stool samples with QIAamp DNA Stool Mini Kit (Qiagen®). DNeasy Blood and Tissues (Qiagen®) kit was applied to the tissue samples according to the manufacturer. The DNA obtained was stored at −20 °C prior to amplification. The F57 insertion sequence was used to detect *Map* DNA. Amplification of the *Map* DNA was performed using the primers F57 (5′-CCTGTCTAATTCGATCACGGACTAGA-3′) and F57Rn (5′-TGGTGTACCGAATGTTGTTGTCAC-3′) (Vansnick et al. 2004). The reactions were prepared in 200-μL microcentrifuge tubes with a final volume of 12.5 μL containing 2.5 μL of genomic DNA, 0.5 μL of each agent specific primer at a concentration of 10 pmol, 2.75 μL of Milli-Q ultrapure water, and 6.25 μL of MasterMix (Qiagen® PCR mixture), according to the manufacturer. The PCRs were performed in a thermocycler (XP Thermal Cycler—Bioer Technology Co. Ltd) using the following steps according to Vansnick et al. (2004): denaturation of the initial DNA at 94 °C for 4 min, 40 cycles at 94 °C for 45 s for denaturation, 68 °C for 45 s for annealing and 72 °C for 45 s for extension, with a final extension at 72 °C for 10 min. Positive controls consisted of previously studied cattle samples, and the negative control consisted of ultrapure water. The analysis of the amplified 424-base pair (bp) product, corresponding to the *Map* DNA was performed by electrophoresis using a 1.5 % agarose gel stained with Blue Green dye (LGC Biotecnologia), which was visualized with ultraviolet light and photographed. We used a standard molecular weight of 100 bp (Fermentas).

Results

Six buffalo herds with paratuberculosis infection were identified, five of which were located in the state of Maranhão and one in Alagoas. All positive herds exhibited clinical signs of the disease characterized by progressive weight loss, submandibular edema (Fig. 1a), foamy dark green liquid diarrhea (Fig. 1b), unresponsiveness to antimicrobial therapy, tenesmus, rough and depigmented coat, dehydration, weakness, and finally death. The body score of the sick buffaloes ranged from 1 to 3 (scale 1–5), which generally segregated from the herd.

Concomitant diseases were identified in these herds, such as infection by gastrointestinal nematodes in adult buffaloes, reproductive disorders, and mastitis. In general, the owners were not familiar with symptomatology of paratuberculosis and things of other causes such as changes in diet, ingestion of foreign bodies, and "worms." After observing buffalo weight loss, some owners sent them to the slaughter house.

The buffaloes had collective access to wetland areas, where they remained crowded for long periods. Several buffalo calves were fed collectively through "communal nursing" by one buffalo cow. The histories provided by the owners unanimously confirm free trade of buffaloes in northeastern Brazil. One common practice in the region was the purchase of groups of lean buffalo from the lowlands of Maranhão for fattening.

From necropsies performed on 26 buffaloes of five farms and two municipal slaughterhouses, 10 of which originating from four farms were affected with paratuberculosis. The clinical signs and lesions were typical and consisted of emaciation, submandibular and cavity edema, thickening of the intestinal wall associated with mucosal roughness (Fig. 2a) and foamy liquid intestinal content, swelling of the mesenteric lymph nodes (Fig. 2b), and mesenteric and intestinal lymphangiectasy. Enteritis (Fig. 2c) and diffuse and pronounced granulomatous lymphadenitis

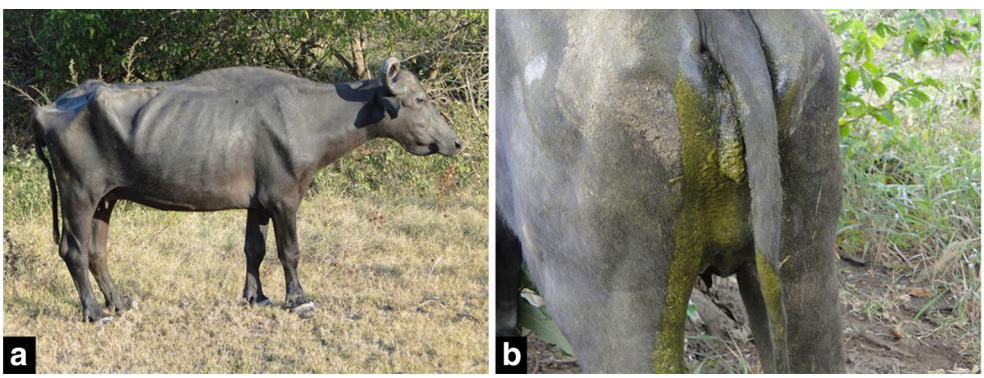

Fig. 1 Buffaloes from Northeastern Brazil with paratuberculosis. **a** Severe weight loss, (body score 1) and submandibular edema. **b** *Dark green* diarrhea

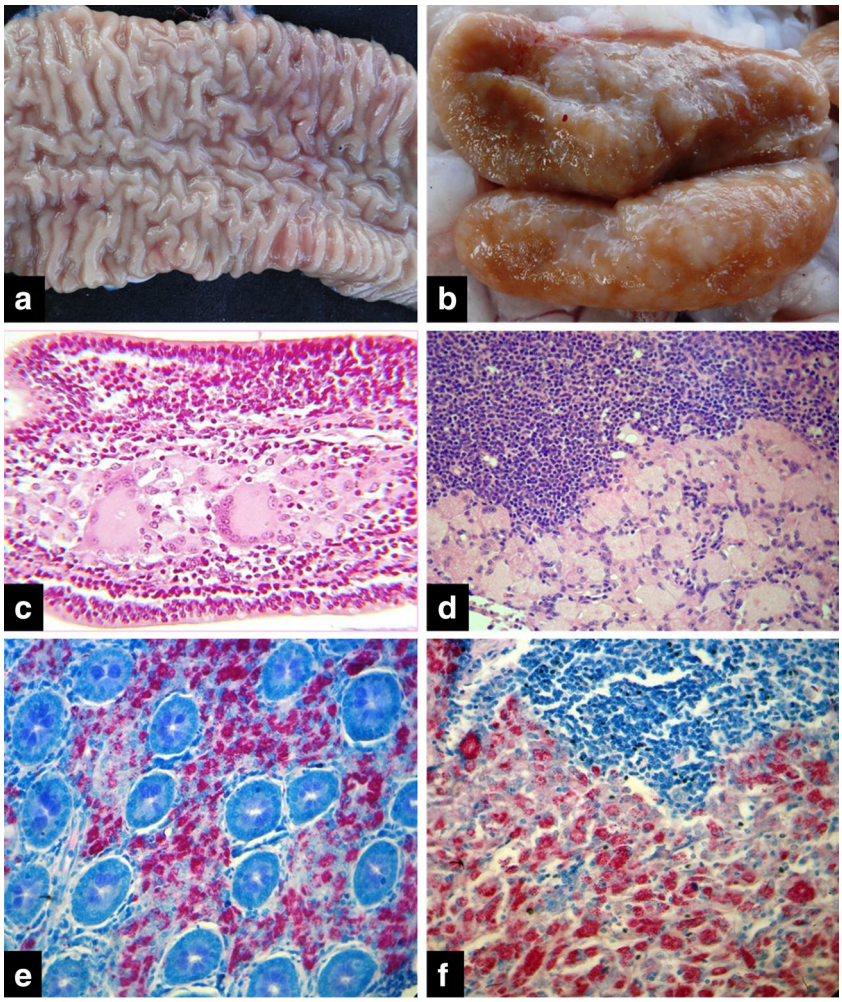

Fig. 2 Buffaloes from Northeastern Brazil with paratuberculosis. **a** Thickened and wrinkled ileal mucosa. **b** Swollen mesenteric lymph node with whitish and irregular areas. **c** Granulomatous enteritis with epithelioid cells and multinucleated giant cells, H&E, Obj.40×. **d** Granulomatous lymphadenitis with a large number of epithelioid cells and multinucleated giant cells, H&E, Obj.25×. **e** Alcohol-acid-resistant bacilli in the small intestinal mucosa, Ziehl-Neelsen, Obj.25×. **f** Alcohol-acid-resistant bacilli in the mesenteric lymph node, Ziehl-Neelsen, Obj.25×

(Fig. 2d) with a large quantity of epithelioid macrophages and multinucleated giant cells were found in those ten buffaloes. In eight buffaloes, Ziehl-Neelsen staining revealed alcohol-acid-resistant bacilli in histologic sections of the intestinal mucosa (Fig. 2e), mesenteric lymph nodes (Fig. 2f) and/or in scrapings of the ileal mucosa (Fig. 3a), in fecal smears (Fig. 3b), and mesenteric lymph node imprints. Moderate hemosiderosis was found in the mesenteric lymph nodes of buffaloes from the five positive herds of Maranhão.

Map DNA was amplified from buffaloes in six of the 16 farms from where originated PCR samples. From those positive farms, two were identified through feces samples by PCR, and four farms were identified through samples taken from feces, the ileum, and mesenteric lymph nodes.

Discussion

Our studies in buffaloes revealed that the dissemination of *Map* is occurring in the same way as it occurs with cattle (Yamasaki et al. 2013b). Resistance to accept the diagnosis of paratuberculosis in the Northeast occurred from several owners of buffaloes herds,

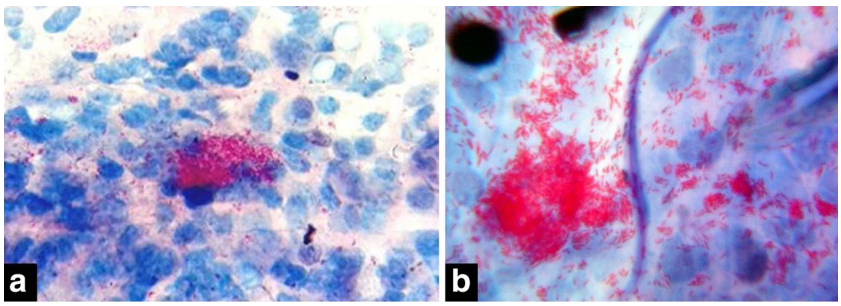

Fig. 3 Buffaloes from Northeastern Brazil with paratuberculosis. **a** Alcohol-acid-resistant bacilli in ileal mucosal scraping, Ziehl-Neelsen, Obj.100×. **b** Alcohol-acid-resistant bacilli in fecal smear, Ziehl-Neelsen, Obj.100×

who did not want to admit that the problem was occurring on their farm nor to accept a visit to investigate the disease. Owners had fear that the information about the disease on their farm would be disseminated; they prefer to slaughter buffaloes in bad conditions. This behavior does not favor the early diagnosis disease in the herd and its control, what is problematic, because paratuberculosis is a chronic disease and difficult to eradicate.

The clinical-pathological picture observed in buffaloes of this study was similar to the one already described by others in the specie (Sivakumar et al. 2006; Mota et al. 2010; Barbosa et al. 2010; Dalto et al. 2012) and in cattle (Driemeier et al. 1999; Mota et al. 2007, 2009; Yamasaki et al. 2013a; Fiss et al. 2015). The differential diagnosis for buffalo paratuberculosis are diseases with diarrhea and weight loss, including gastrointestinal nematodes, non-infectious diarrhea, and nutritional deficiency diseases. As observed by Belo-Reis et al. (2016), this study revealed buffaloes with rough and depigmented coat and hemosiderosis of lymph nodes, indicating copper deficiency.

Action should be taken to control paratuberculosis by eliminating ruminants in clinical stage of the disease. The adoption of good health management practices has been found to be most effective for control of the disease (Brito et al. 2014). Vaccine is being developed with live-attenuated strains of *Map*, and genomics and bioinformatics are promising research areas for future control of the disease (Bannantine and Talaat 2015).

Collective use of wetlands by buffaloes and the contamination of water and food with infected feces as well as the use of "communal nursing" favor the spread of *Map* (Dalto et al. 2012). According to the literature, calves rarely eliminate *Map*, so that calf-to-calf transmission is less important. However, a recent study showed *Map* excretion in feces by calves, strengthening the hypothesis of transmission through calves in dairy cattle farms (Wolf et al. 2015).

The little knowledge about the disease, free trade of animals, and lack of a control program favor the spread of *Map* and contribute to an increasing number of affected herds. It is important to warn buyers of the risk to consume contaminated products, given the zoonotic potential of *Map* and its likely relationship with Crohn's disease (Atreyaa et al. 2014).

Diagnosis of Johne's disease has been established in buffaloes in Maranhão and Pernambuco (Barbosa et al. 2010; Mota et al. 2010), in dairy cattle in Pernambuco (Mota et al. 2007) and Paraíba (Oliveira et al. 2008; Mota et al. 2009; Medeiros et al. 2012b), as well as in sheep and goats in Paraíba (Oliveira et al. 2010; Medeiros et al. 2012a). This study reveals that similar to its spread to other ruminant species, paratuberculosis is spreading in buffaloes of Northeast Brazil. Longitudinal and continuous data of high quality obtained are essential to understand the pathobiology and epidemiology of *Map* infection in herds (Schukken et al. 2015). This underscores the importance of studies aimed to control paratuberculosis, particularly to identify subclinical animals.

Acknowledgments The authors would like to thank the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq) for the financial support while performing the postdoctoral scholarship senior (PDS) via Call Notice CNPq/MAPA/SDA no. process 150259/2013-1, the veterinarians Mauro Gonçalves, Débora Rochelli, and André Santos for their support during the work, and to Letícia Pinto for helping with the laboratory tests.

Compliance with ethical standards

Statement of animal rights All the rural producers were informed about the utilization of their animals/samples during this investigation, consent was obtained, and the investigation adhered to a high standard of veterinary care.

Conflict of interest The authors declare that they have no conflict of interest.

References

Atreyaa, R., Bülteb, M., Gerlach, G.F., Goethed, R., Hornef, M.W., Köhler, H., Meens J., Möbius, P., Roeb, E. and Weiss, S. 2014. Facts, myths and hypotheses on the zoonotic nature of *Mycobacterium avium* subspecies *paratuberculosis*, International Journal of Medical Microbiology, 304, 858–867

Bannantine, J.P. and Talaat, A.M. 2015. Controlling Johne's disease: vaccination is the way forward, Frontiers in Cellular and Infection Microbiology, 5:1–2

Barbosa, J.D., Oliveira, C.M.C., Silveira, J.A.S., Albernaz, T.T., Silva, N.S., Reis, A.S.B., Oliveira, C.H.S. and Yamasaki, E.M. 2010. Paratuberculosis in water buffaloes (*Buballus bubalis*) in Maranhão State, Brazil. In: Proceedings of the 26th World Buiatrics Congress, Santiago, Chile, 334

Bartlett, J.H. 2013. Microorganisms. In: Suvarna, S.K., Layton, C., and Bancroft, J.D. (eds), Bancroft's Theory and Practice of Histological Techniques, 17th ed. (Churchill Livingstone Elsevier, London), 296–298

Belo-Reis, A.S., Brito, M.F., Bomjardim, H.A., Faial, K.C.F., Salvarani, F.M., Ubiali, D.G., Oliveira, C.M.C. and Barbosa J.D. 2016. Teores de cobre, zinco e ferro no fígado de búfalos (*Bubalus bubalis*) com paratuberculose, Pesquisa Veterinária Brasileira, 36, 24–28

Brito, M.F., Mota, R.A. and Yamasaki, E.M. 2014. Paratuberculose: perguntas e respostas. Universidade Federal Rural de Pernambuco. http://institucional.ufrrj.br/sap/files/2014/12/cartilha-PARATUBERCULOSE-Perguntas-e-Respostas-out-2014.pdf

Dalto, A.C., Bandarra, P.M., Pavarini, S.P., Boabaid, F.M., Bitencourt, A.P.G., Gomes, M.P., Chies, J., Driemeier, D. and Cruz, C.E.F. 2012. Clinical and pathological insights into Johne's disease in buffaloes, Tropical Animal Health and Production, 44:1–5

Driemeier, D., Cruz, C.E.F., Gomes, M.J.P., Corbellini, L.G., Loretti, A.P. and Colodel, E.M. 1999. Aspectos clínicos e patológicos da paratuberculose em bovinos no Rio Grande do Sul, Pesquisa Veterinária Brasileira, 19, 109–115

Fiss, L., Santos, B.L., Albuquerque, P.P.F., Mota, R.A., Marcolongo-Pereira, C., Adrien, M.L., Soares, M.P. and Schild, A.L. 2015. Paratuberculose em bovinos de corte na região Sul do Rio Grande do Sul, Pesquisa Veterinária Brasileira, 35, 437–442

Medeiros, J.M.A., Garino Jr, F., Almeida, A.P., Lucena, E.A. and Riet-Correa, F. 2012a. Paratuberculose em ovinos e caprinos no estado da Paraíba, Pesquisa Veterinária Brasileira, 32, 111–115.

Medeiros, J.M.A., Garino Jr, F., Matos, R.A.T., Costa, V.M.M. and Riet-Correa, F. 2012b. Frequência de anticorpos para paratuberculose em bovinos no semiárido paraibano, Pesquisa Veterinária Brasileira, 32, 697–700

Mota, R.A., Pinheiro-Junior, J.W., Gomes, M.J.P., Peixoto, R.M., Maia, C.L., Brito, M.F., Chies, J.A.B., Snel, G.G.M., Bercht, S. and Juffo, G.D. 2007. Paratuberculose em um rebanho leiteiro no Estado de Pernambuco, PE, Arquivos do Instituto Biológico, 74, 73–79

Mota, P.M.P.C., Pires, P.S., Assis, R.A., Salvarani, F.M., Leite, R.M.H., Dias, L.D., Leite, R.C., Lobato, F.C.F., Guedes, R.M.C. and Lage, A.P. 2009. Paratuberculosis in a dairy Gyr herd in a state of Paraíba, Brazil. Pesquisa Veterinária Brasileira, 29, 703–706

Mota, R.A. Peixoto, P.V., Yamasaki, E.M., Medeiros, E.S., Costa, M.M., Peixoto, M.P., Brito, M.F. 2010. Ocorrência de paratuberculose em búfalos (*Bubalus bubalis*) em Pernambuco, Pesquisa Veterinária Brasileira, 30, 237–242

Oliveira, D.M., Pimentel, L.A., Rodrigues, T.A., Dantas, A.F.M., Miranda-Neto, E.G., Simões, S.V.D. and Riet-Correa, F. 2008. Paratuberculose em bovinos no Estado da Paraíba. In: Proceedings of the 5th Encontro Nacional de Diagnóstico Veterinário, Campo Grande, Brasil

Oliveira, D.M., Riet-Correa, F., Galiza, G.J.N., Assis, A.C.O., Dantas, A.F.M., Bandarra, P.M. and Garino Jr, F. 2010. Paratuberculose em caprinos e ovinos no Brasil, Pesquisa Veterinária Brasileira, 30, 67–72

Schukken, I.H., Whitlock, R.H., Wolfgang, D., Grohn, Y., Beaver, A., Vankessel, J., Zurakowski, M. and Mitchell, R. 2015. Longitudinal data collection of *Mycobacterium avium* subspecies *paratuberculosis* infections in dairy herds: the value of precise field data, Veterinary Research, 46, 65–76

Sivakumar, P., Tripathi, B.N., Singh, N. and Sharma, A.K. 2006. Pathology of naturally occurrence paratuberculosis in water buffaloes (*Bubalus bubalis*), Veterinary Pathology, 43, 455–462

Vansnick, E., Rijk, P., Vercammen, F., Geysen, D., Rigouts, L. and Portaels, F. 2004. Newly developed primers for the detection of *Mycobacterium avium* subspecies *paratuberculosis*, Veterinary Microbiology, 100, 197–204

Wolf, R., Orsel, K., De Buck, J. and Barkema, H.W. 2015. Calves shedding *Mycobacterium avium* subspecies *paratuberculosis* are common on infected dairy farms, Veterinary Research, 46, 71–79

Yamasaki, E.M., Brito, M.F., McIntosh, D., Galvão, A., Peixoto, T.C. and Tokarnia C.H. 2013a. Diagnóstico imuno-histopatológico da paratuberculose subclínica em bovinos no estado do Rio de Janeiro, Pesquisa Veterinária Brasileira, 33, 1427–1432

Yamasaki, E.M., Brito, M.F., Mota, R.A., McIntosh, D. and Tokarnia, C.H. 2013b. Paratuberculose em ruminantes no Brasil, Pesquisa Veterinária Brasileira, 33, 127–140