Epidemiological investigation of paratuberculosis in buffaloes and cattle in the state of Pará, Brazil

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Investigação epidemiológica de paratuberculose em búfalos e bovinos no estado do Pará, Brasil

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Abstract

Paratuberculosis is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The objective of this research was to detect the presence of antibodies against MAP, through an indirect ELISA, in blood serum from cattle and buffaloes belonging to the state of Pará, Brazil. A total of 1,772 samples were tested, 1,190 from cattle from 78 herds, from 41 municipalities and 582 from buffaloes, from 36 herds, in 13 municipalities. To compare the results obtained between the species evaluated, the contingency Chi-square test was applied, to test the hypothesis of the existence of an association between positive and negative in relation to the species, for this purpose, the level of significance adopted was equal to 0.05. The total seropositivity found was 31.04% (550/1,772). Of the buffalo samples, 89 were positive (15.29%) and 493 negative (84.71%), in relation to the 13 municipalities evaluated, 11 had reactive animals. In relation to cattle, 461 were reactive (38.73%) and of the 41 municipalities analyzed, 40 had seropositive animals. The distribution of positive and negative cases between species indicates that of the total of 1,190 cattle evaluated, 38.74% of the samples were positive, while of the total of 582 buffaloes, this percentage corresponded to 15.29%. There was a significant association between test results and species, with a higher percentage of positive cases in cattle ($p < 0.01$). MAP infection is widely spread among cattle and buffaloes in the state of Pará. Furthermore, differences in prevalence between them suggest lower susceptibility of buffaloes compared to cattle.

Keywords: *Mycobacterium avium*. Indirect ELISA. MAP.

Resumo

A paratuberculose é causada pelo Mycobacterium avium *subsp. paratuberculosis (MAP). O objetivo desta pesquisa foi detectar a presença de anticorpos contra MAP, através de um ELISA indireto, no soro sanguíneo de bovinos e búfalos pertencentes ao estado do Pará, Brasil. Foram testadas 1.772 amostras, sendo 1.190 de bovinos de 78 rebanhos, de 41 municípios e 582 de búfalos, de 36 rebanhos, em 13 municípios. Para comparar os resultados obtidos entre as espécies avaliadas, aplicou-se o*

teste qui-quadrado de contingência, para testar a hipótese da existência de associação entre positivos e negativos em relação às espécies, para tanto, o nível de significância adotado foi igual a 0,05. A soropositividade total encontrada foi de 31,04% (550/1.772). Das amostras de búfalos, 89 foram positivas (15,29%) e 493 negativas (84,71%), em relação aos 13 municípios avaliados, 11 apresentaram animais reagentes. Em relação aos bovinos, 461 foram reativos (38,73%) e dos 41 municípios analisados, 40 tiveram animais soropositivos. A distribuição dos casos positivos e negativos entre as espécies indica que do total de 1.190 bovinos avaliados, 38,74% das amostras foram positivas, enquanto do total de 582 búfalos, esse percentual correspondeu a 15,29%. Houve associação significativa entre os resultados dos testes e a espécie, com maior percentual de casos positivos em bovinos (p < 0,01). A infecção por MAP está amplamente disseminada entre bovinos e bubalinos no estado do Pará. Além disso, as diferenças de prevalência entre eles sugerem menor suscetibilidade dos búfalos em relação aos bovinos.

Palavras-chave: Mycobacterium avium. *ELISA indireto. MAP.*

Introduction

Paratuberculosis or Johne's disease, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a chronic granulomatous enteritis that affects ruminants and many other species of domestic animals (Santana et al., 2022) and is characterized by persistent diarrhea, weight loss and protein enteropathy, eventually followed by death (Cheng et al., 2020).

Most cattle are infected from calves through ingestion of feces, milk or water contaminated with MAP (Steuer et al., 2021). The long incubation period occurs due to MAP excretion in feces for months and even years before clinical symptoms develop (Roller et al., 2020).

Johne's disease causes economic losses worldwide for livestock farmers and related industries, in terms of milk and meat production (Liening-Ewert et al., 2023). In dairy and beef herds, losses are associated with reduced milk production and weight gain, lower reproductive efficiency, early culling, and reduced values of slaughtered cattle (Ozsvari et al., 2020).

Knowledge and understanding of the epidemiology of MAP has been hampered for many years due to the inability to discriminate MAP from environmental species of *M. avium* and to differentiate MAP isolates from different host species and different geographic locations (Imada et al., 2023).

Thus, recent molecular biology studies have led to the improvement of techniques with sufficient capacity to differentiate subspecies of *M. avium* (Pickrodt et al., 2023), such as typing based on mobile, repetitive genetic elements, and single nucleotide pleomorphism, SNPs, of MAP (Elsohaby et al., 2021).

The isolation of the agent in the feces or tissues of infected animals is considered the gold test for diagnosis, but due to the lack of qualifications in veterinary bacteriology and the difficulties inherent in the cultivation of MAP, it is rarely performed in Brazil (Husakova et al., 2020). Therefore, the enzyme-linked immunosorbent assay (ELISA) is currently being used as a diagnostic test due to its practicality, low cost, providing the analysis of many samples at the same time (Whittington et al., 2019).

Several studies point to a significant and concrete association between infection by MAP and Crohn's disease in humans (Okuni et al., 2020), which leads to chronic granulomatous ileocolitis, characterized by diarrhea, fever and intermittent abdominal pain (Kuenstner et al., 2020). The transmission process may be associated with the consumption of raw or inadequately pasteurized milk (Agrawal et al., 2021).

In view of the above, the importance of studying the occurrence of MAP is evident and therefore the objective of this research was to detect the presence of antibodies, through a commercial ELISA against MAP, in blood serum from cattle and buffaloes belonging to the state of Pará, Brazil.

Material and methods

This research received approval from the Ethics Committee on the Use of Animals at the Federal University of Pará, protocol 9600280616).

To carry out the work, 1,772 blood serum samples were tested, 1,190 of which came from cattle grouped in 78 herds, distributed in 41 municipalities in the state and 582 from buffaloes originating from 36 herds located in 13 municipalities. The samples were collected from adult animals over three years

of age with no history of health problems. Collection took place at two locations: slaughterhouses, where blood was obtained during bleeding, and farms, where jugular venipuncture was performed. In both cases, tubes with clot activator gel were used.

After collection, the tubes were kept upright at room temperature for approximately 30 minutes to allow the clot to retract. The samples were then packed in an insulated box with 3 to 4 units of recyclable ice (400 g each) for transport, ensuring that the interval between collection and serum freezing was less than 24 hours.

The serum was separated by centrifugation (1,500 rpm/10 min), transferred to Eppendorf-type tubes, and frozen at -20 °C. The samples remained frozen until the time of analysis, when they were thawed exclusively for testing using the commercial ELISA kit.

Serological analysis

The serum samples were analyzed using the commercial indirect ELISA kit Screen® Paratuberculosis Indirect ELISA kit-Screening Format, for detection of anti-MAP antibodies in bovine serum. The test was carried out according to the manufacturer's recommendations (SVANOVIR®). Conjugates were prepared with peroxidase-conjugated monoclonal antibodies. The substrate used was tetramethylbenzidine and OPD (O-phenylenediamine, Sigma Chemical Company-USA) was used as the chromogen. The reading was taken at a wavelength of 450 nm, which was transformed into a percentage of sera/positives (S/P%).

Results were calculated as the sample-to-positive control ratio (S/P), derived by subtracting the mean negative-control OD value from each sample and dividing this by the corrected positive-control OD value (i.e., the mean positive-control OD minus the mean negative-control OD). The sample ODs were then compared with the kit positive-control OD to derive S/P ratios. The cut-off point for samples considered negative for anti-MAP antibodies was S/P values equal to or less than 60%. For suspected samples, the cut-off point was between 60% and 70%, and S/P values equal to or greater than 70% were considered positive. A sample with an S/P ratio of ≥ 0.30 was considered positive for *Mycobacterium bovis* antibodies according to the manufacturer's recommendations. The geographic dis-tribution of MAP-seropositive buffaloes and cattle by municipality is shown in Figure 1.

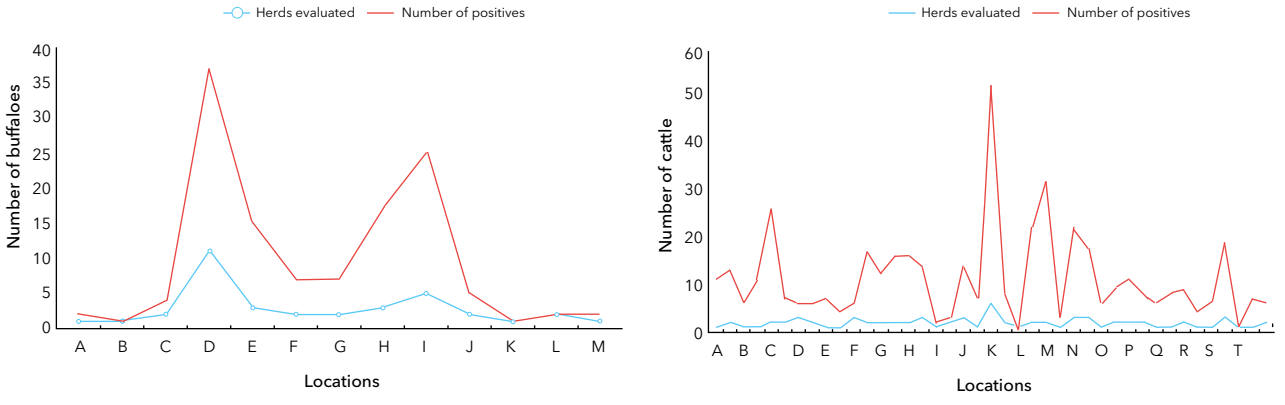

Figure 1 - Seroprevalence of *Mycobacterium avium* subsp. *paratuberculosis* in buffaloes (n = 582) and cattle (1,190) by municipality of origin.

Note: Buffaloes locations: A = Belém; B = Bujaro; C = Cachoeira do Arari; D = Chaves; E = Mojú; F = Peixe-Boi; G = Ponta de Pedras; H = Salvaterra; I = Santa Cruz do Arari; J = Santa Isabel do Pará; K = Soure; L = Terra Alta; M = Vigia.

Note: Cattle locations: A = Altamira; B = Bannach; C = Benevides; D = Bom Jesus do Tocantins; E = Capanema; F = Chaves; G = Dom Elizeu; H = Ipixuna do Pará; I = Itaituba; J = Monte Alegre; K = Ponta de Pedras; L = Rio Maria; M = Salvaterra; N = Santa Isabel do Pará; O = Santo Antônio do Tauá; P = São Félix do Xingú; Q = São Miguel do Pará; R = Tailândia; S = Tomé Açu; T = Ulianópolis.

Statistical analysis

To compare the results obtained between the species evaluated, the contingency Chi-square test was applied, in order to test the hypothesis of the existence of an association between positive and negative in relation to the species, for this purpose, the level of significance adopted was equal to 0.05.

Results

The prevalence of MAP infection was 31.04% (550/1,772). Of the buffalo samples, 89 were positive (15.29%) and the number of herds with at least one positive animal was 88.88% (32/36) and of 13 municipalities evaluated, 11 had reactive animals (Figure 2). The percentage of positivity ranged from 4.34 to 27.27% and the municipalities of Bujaru, Soure and Terra Alta did not present herds with reactive buffaloes. In the analysis of bovine sera, 461 were reactive (38.73%) and of the 41 municipalities investigated, 40 had seropositive animals (Figure 3). The frequency of reactive cattle ranged from 10 to 77.77%, being the municipalities of Ulianópolis (77.77%), Rio Maria (73.33%), Acará (73.33%), Bannach (68.75%) and Rondon do Pará (64%) which had positive animals on the properties analyzed.

In the analysis of cattle, 461 were reactive (38.73%) and of the 41 municipalities investigated, 40 had seropositive animals (Figure 3). The frequency of reactive cattle ranged from 10 to 77.77%, being the municipalities of Ulianópolis (77.77%), Rio Maria (73.33%), Acará (73.33%), Bannach (68.75%) and Rondon do Pará (64%) which had positive animals on the properties analyzed.

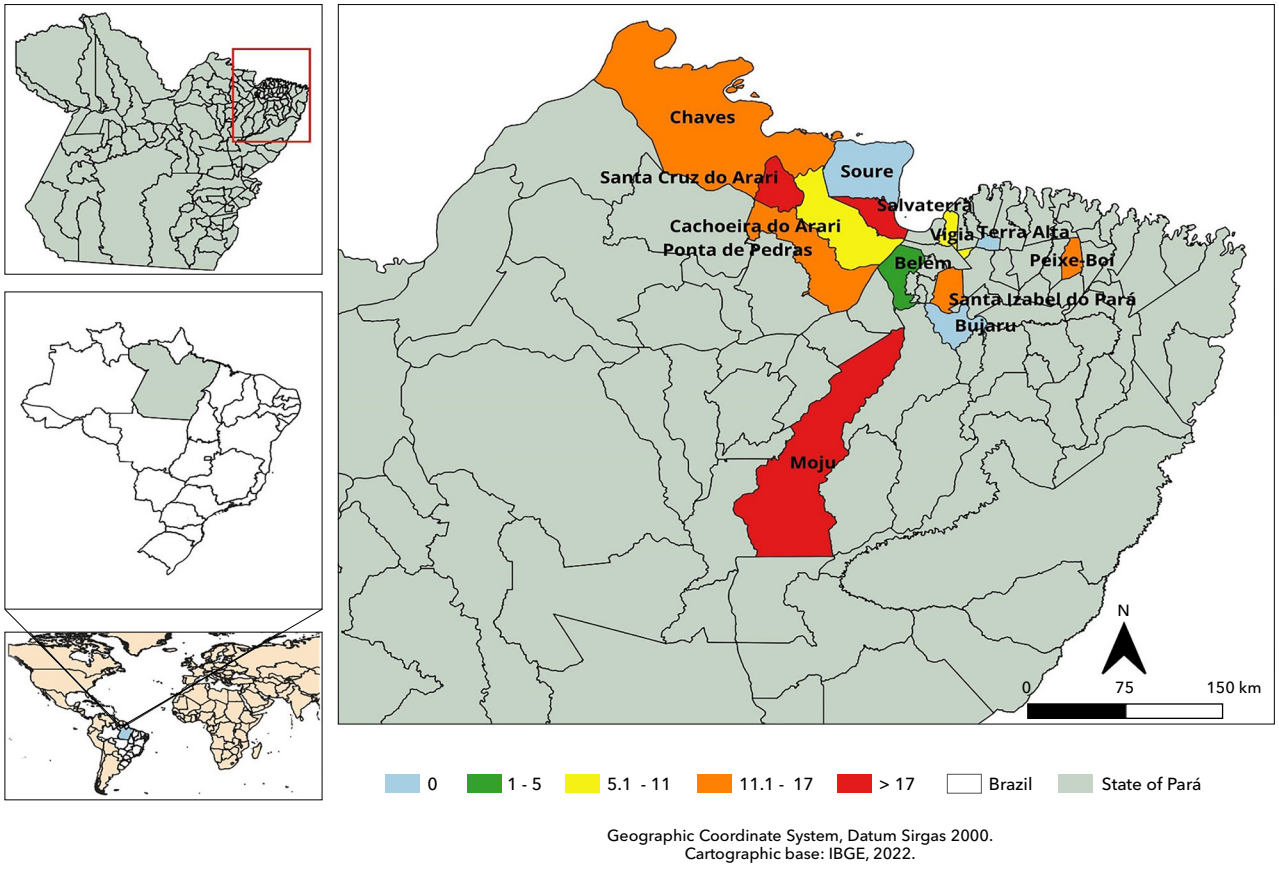

Figure 2 - Seroprevalence of *Mycobacterium avium* subsp. *paratuberculosis* in buffaloes by municipality of origin in Pará, Brazil (n = 582). Percentage values range from 4.34 to 27.27%.

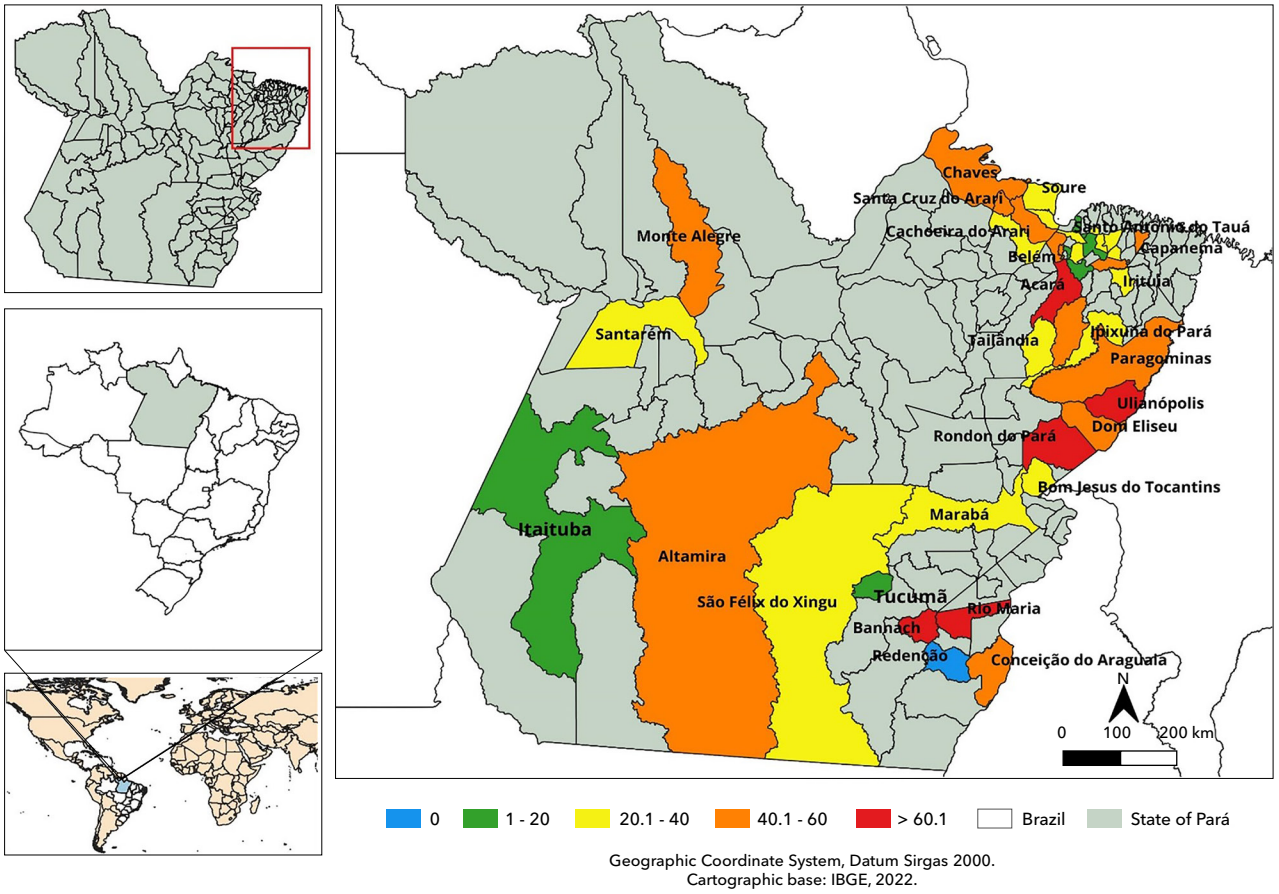

Figure 3 - Seroprevalence of *Mycobacterium avium* subsp. *paratuberculosis* in cattle by municipality of origin in Pará, Brazil (n = 1,190). Percentage values range from 10 to 77.77%.

The distribution of MAP seropositivity by species is shown in Table 1. Cattle had a significantly higher seroprevalence (38.73%; 461/1,190) compared to buffaloes (15.29%; 89/582), with an overall prevalence of 31.04% (550/1,772). This difference between species was statistically significant ($\chi^2 = 10.39$; $p < 0.0001$).

Table 1 - Number and percentage of MAP-seropositive and seronegative samples by species detected through indirect ELISA

Species	Positive	Negative	Total
Cattle	461 (38.73%)	729 (61.27%)	1,190 (67.16%)
Buffaloes	89 (15.29%)	493 (84.71%)	582 (32.64%)
Total	550 (31.04%)	1,222 (68.96%)	1,772 (100%)

Note: MAP = *Mycobacterium avium* subsp. *paratuberculosis*. Chi-square = 10.39 ($p < 0.0001$).

Discussion

Based on the results obtained, MAP infection is widespread in the Pará state. In cattle, the positivity found (38.73%) was present in 40/41 municipalities evaluated. This result is close to Barbosa et al. (2023) and higher than that found by Piovesan (2021) who reported seropositivity of 35.4 and 0.8%, for the states of Pará and Rio Grande do Sul, respectively.

Still in Rio Grande do Sul, Setim et al. (2023) carried out a prospective, cross-sectional, and observational study on dairy cattle from commercial farms in five mesoregions of the state. The samples were tested with indirect ELISA, of the 362 cows tested, 20 were seroreactive, suggesting that paratuberculosis is probably endemic in the northwest and northeast mesoregions of that state.

In Paraná, the presence of antibodies against MAP was investigated, using ELISA, in high-producing dairy

cows using blood and fecal samples collected from 708 animals, from 54 herds, over 2 years, from five counties. The results found by Camilo et al. (2022) were much higher than those in this research, as the prevalence of antibodies against MAP found by them in the herd was 61.1%, varying from 12.5 to 80% between municipalities, and the prevalence in animals was 9.8%; ranging from 0 to 87.5% per herd.

In Chile, a random sample of 40 dairy herds with a previous history of MAP infection was selected, totaling 4,963 animals. The average prevalence apparent in the research developed by Verdugo et al. (2020) was 6.3% and all sampled herds had seropositive animals. This result was higher than that found in this research, since one of the herds evaluated did not present seropositives.

In this research, the occurrence of infection in buffaloes was lower in all aspects: animal, herd, and municipality, in relation to cattle. There are few studies on the occurrence of MAP in buffaloes in Brazil, with Albuquerque et al. (2018), the most recent and the first to carry out a molecular epidemiological study of MAP in Brazil, which concluded that paratuberculosis is widespread in cattle and buffaloes, in several regions of Brazil.

Two years earlier, Brito et al. (2016) investigated several farms in Northeast Brazil to identify the occurrence of paratuberculosis in buffaloes. The samples were obtained from 17 farms, two slaughterhouses and a quarantine area in the Northeast. Of these, six farms were positive for Johne's disease, indicating that the disease is widespread throughout the Brazilian Northeast. Before that, in the state of Rio Grande do Sul, Dalto et al. (2012) described a buffalo herd with 203 animals, which presented 21 infected with MAP and seven animals with clinical symptoms (Yamasaki et al., 2013).

Research on the occurrence of MAP infection in buffaloes in Brazil is recent and scarce, with the first occurrence reported in 2010, by Mota et al. (2010) from a herd of 100 animals in Pernambuco. Of these, five showed characteristic clinical signs of the disease, direct examination using the Ziehl-Neelsen technique to search for the bacillus in fecal smears, intestinal mucosa scrapings and imprints of mesenteric lymph nodes resulted positive, as well as the IS900 PCR specific to mesenteric lymph nodes and mucosa.

In the state of Pará, Barbosa et al. (2023) reported the first case of clinical paratuberculosis, in which necropsy revealed thick and wrinkled intestinal mucosa, as well as enlarged lymph nodes. This indicates the presence of the disease in the region and corroborates what Silva (2005) highlighted almost two decades ago, namely the high number of animals with anti-MAP antibodies.

The widespread MAP infection in Pará demands integrated control strategies. Recent studies (Idris et al., 2021; Rasmussen et al., 2021a,b; Griss et al., 2024) have evaluated several factors and revealed that economic analyses demonstrate that vaccination with dual-effect vaccines is the most cost-effective approach, with benefit-cost ratios of 1.48 to 2.13, while testing and culling remains economically unviable in most regions. The economic burden is substantial, with infected cattle experiencing median milk yield reductions of 452 kg/lactation, prolonged calving intervals of approximately 30 days, and culling rates 1.5 to 3 times higher than uninfected animals. In Pará, a multifaceted approach combining targeted surveillance, biosecurity measures, and selective vaccination may offer the most practical pathway to reduce MAP prevalence and mitigate production losses.

Conclusion

Based on the results found, it is suggested that the differences in prevalence between cattle and buffaloes mean less susceptibility of these compared to the former, regarding paratuberculosis. MAP infection is widespread in the state of Pará, and in addition to cattle, buffaloes are also affected, albeit to a lesser extent. Finally, given the results obtained by several studies in Brazil, combined with those described here, attention must be paid to the danger of spreading paratuberculosis in cattle and buffalo herds.

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Authors´contributions

RNCCJ was responsible for the study conception and design; EBRS, for data acquisition, analysis, and interpretation; RNCCJ, CVA and EBRS, for data collection, analysis, and interpretation; and CVA, for statistical analysis. HLTD effectively participated in the research supervision and, with WCS, in the critical literature review. All authors contributed to the manuscript writing, critical revision of important intellectual content, and approval of the final version of the manuscript.

Data availability statement

The research data are not publicly available.

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