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Seroprevalence of Mycobacterium avium subsp. paratuberculosis in quarantined cattle in Rio Grande do Sul, Brazil (2019 to 2023)

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Research Article

Keywords: ELISA, Export diagnosis, Mycobacterium avium subsp. paratuberculosis, Seroprevalence, Zoonosis

Posted Date: November 25th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11651418/v1

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Abstract

Bovine paratuberculosis (BPTB), also known as Johne's disease, is a chronic intestinal disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The main clinical signs include progressive weight loss and chronic diarrhea, negatively impacting animal production and health. BPTB has a significant economic impact on livestock due to reduced productivity and increased costs associated with disease control and treatment. In addition, the disease has potential zoonotic importance, being associated with Crohn's disease in humans, with possible transmission through contaminated meat and dairy products. In Brazil, epidemiological and seroprevalence studies of BPTB are limited, with only isolated reports from several states. This study investigated the seroprevalence of antibodies against MAP in cattle quarantined for export in the state of Rio Grande do Sul between 2019 and 2023. A total of 30,247 serum samples were tested with an indirect enzyme-linked immunosorbent assay (ELISA), and distinct seroprevalences were observed across mesoregions: Southeast (0.52%), Central-Western (0.48%), Metropolitan (0.40%), Southwest (0.34%), Northeast (0.29%) and Northwest (0.24%). In the Central-Eastern mesoregion, no sample presented positive results. The observed seroprevalence can be attributed to the characteristics of the herds evaluated, predominantly beef cattle raised in an extensive system, where contact between animals is limited, reducing the spread of the agent. These findings enhance our understanding of BPTB epidemiology in the context of live cattle export from Brazil and underscore the need for sustained surveillance and targeted control strategies to reduce the economic and public health risks associated with the disease.

Introduction

The livestock sector in Brazil has one of the largest cattle herds in the world, totaling 234.4 million head in 2022 (IBGE 2023). Between 2017 and 2019, the country exported approximately 1.5 million live cattle, generating over one billion US dollars in revenue. The state of Rio Grande do Sul (RS) alone contributed nearly 23% of the exported animals (MDIC 2026).

To ensure compliance with export requirements, companies adhere to disease testing protocols mandated for the issuance of the International Zoosanitary Certificate (IZC), which vary depending on the importing country. For example, exports to Turkey require screening for paratuberculosis (PTB), bovine viral diarrhea (BVD), enzootic bovine leukosis (EBL), tuberculosis, and brucellosis, among other diseases (MAPA 2021).

Bovine paratuberculosis (BPTB), also known as Johne's disease, is a chronic contagious intestinal disease that primarily affects ruminants, although other species may also develop the illness. The etiological agent is *Mycobacterium avium* subsp. *paratuberculosis* (MAP), an obligate intracellular, Gram-positive and acid-fast bacterium whose acid-fastness is due to its lipid-rich cell wall (McVey et al. 2013; Correa-Valencia et al. 2018). The primary route of transmission is fecal-oral, although transplacental and transmammary transmission can also occur. Calves between 0 and 6 months of age are the most susceptible, and the incubation period can range from six months to over 15 years (Correa-Valencia et al. 2018).

The main clinical signs of this disease include chronic diarrhea and progressive weight loss, leading to significant economic losses in affected herds. Infected animals may exhibit decreased productivity, lower reproductive performance, and higher susceptibility to secondary infections such as mastitis (Setim et al. 2023a).

Paratuberculosis is included in the list of multiple-species diseases, infections and infestations of the World Organisation for Animal Health (WOAH, formerly OIE; OIE 2022). The relevance of BPTB to public health is due to its potential zoonotic link to Crohn's disease in humans (Waddell et al. 2016). Milk and dairy products are among the potential vehicles of human exposure, and viable MAP has been recovered from commercially pasteurized milk (Waddell et al. 2016; Kuenstner and Kuenstner 2021).

Diagnosis of PTB involves either direct detection of the pathogen or assessment of the host's immune response (Manning and Collins 2001). Common techniques include fecal culture, immunological assays targeting humoral or cellular responses, polymerase chain reaction (PCR)-based molecular tests, and histopathology (Roussel et al. 2007). Therefore, this study aimed to evaluate the seroprevalence of anti-MAP antibodies in cattle held in export quarantine facilities in Rio Grande do Sul from 2019 to 2023.

Materials and methods

Animals and study area

This study was based on the retrospective analysis of bovine serum samples collected during routine quarantine procedures. The samples were obtained at the Pre-Embarkation Establishment (PEE) in Capão do Leão, RS (coordinates − 31.770933, − 52.417123) during the years 2019, 2020, 2022, and 2023. No samples were collected in 2021, as no export operations to Turkey took place that year. At the PEE, animals from various regions of the state were received and subjected to health procedures in accordance with the requirements of the respective IZC for each importing country. The samples analyzed were obtained from non-castrated European breed cattle, aged 0–12 months and weighing 200–250 kg. Individual data on the establishments of origin of the animals were obtained through a traceability system (Data Link Tru-Test software, Tru-Test, Porto Alegre, Brazil) and exported to a Microsoft Excel spreadsheet.

Diagnosis of paratuberculosis

Serum samples were stored in microtubes (Eppendorf) and refrigerated at 4°C until processing. For diagnosis, an indirect enzyme-linked immunosorbent assay (ELISA) was performed using the commercial Paratuberculosis Indirect Screening Test (IDVET, Montpellier, France), following the manufacturer's instructions. The microplates were pre-coated with the PTB antigen.

In a sterile 96-well microplate (CRAL), 110 µl/well of dilution buffer was added. Negative controls (10 µl) were placed in wells A1 and B1, and positive controls in wells C1 and D1. In each of the remaining wells, 10 µl of the serum sample to be tested was added. Plates were incubated for 45 ± 4 minutes at 23°C. After this initial incubation, the contents were transferred to the pre-coated plates and incubated again for 45 minutes at 21 ± 5°C. Plates were then emptied and washed three times with the washing solution provided in the kit.

Subsequently, 100 µl of a solution containing anti-bovine IgG conjugated with horseradish peroxidase (HRP) (1×) was added to bind anti-MAP antibodies. The plates were incubated for 30 minutes at 23°C, followed by removal of the conjugate and three additional washes. A substrate solution (100 µl/well) was then added and incubated for 15 minutes at 37°C. The reaction was stopped by adding a stop solution.

The plates were read using a spectrophotometer, and the optical density was measured at a wavelength of 450 nm (OD450). The sample-to-positive control ratio (S/P) was used to interpret the results: S/P = [(OD450 sample − OD450 negative control) / (OD450 positive control − OD450 negative control)] × 100.

According to the manufacturer's guidelines, serum samples were considered negative for anti-MAP antibodies when S/P values were equal to or less than 60%, suspect when S/P values were between 60% and 70%, and positive when S/P values were equal to or greater than 70%.

All samples were processed at the veterinary diagnostic laboratory annexed to Agropecuária Machado, located in Pelotas, Rio Grande do Sul.

Statistical analysis

The serology results (positive and negative) were recorded in Microsoft Excel, and the seroprevalence for each place of origin was calculated using the formula: (number of positive samples / total number of samples) × 100.

To characterize seroprevalence, the state of RS was divided into mesoregions according to the classification of the Brazilian Institute of Geography and Statistics (IBGE): (i) Central-Western; (ii) Central-Eastern; (iii) Metropolitan; (iv) Northeast; (v) Northwest; (vi) Southeast; and (vii) Southwest.

Seroprevalence over the whole study period was mapped by mesoregion, with the value of each mesoregion shown in the legend, and annual seroprevalence was mapped in five classes (0–0.16%, 0.16–0.33%, 0.33–0.49%, 0.49–0.66%, and > 0.66%). The maps were generated in QGIS (QGIS Association, https://www.qgis.org).

Results

Between 2019 and 2023, a total of 30,247 bovine serum samples from all regions of RS were analyzed. Over the four sampling years, the highest seroprevalence was observed in the Southeast mesoregion (0.52%), followed by the Central-Western (0.48%), Metropolitan (0.40%), Southwest (0.34%), Northeast (0.29%), and Northwest (0.24%) mesoregions. No positive samples were detected in the Central-Eastern mesoregion (Fig. 1).

On an annual basis, the highest regional seroprevalence was observed in the Northeast mesoregion in 2022 (0.95%). In 2023, seroprevalence declined in most mesoregions compared with previous years (Fig. 2).

Discussion

Bovine paratuberculosis is a chronic granulomatous enteritis that affects domestic and wild ruminants, caused by MAP. The disease leads to significant economic losses due to decreased productivity, increased culling rates, and veterinary care expenses (Manning and Collins 2001). For instance, in the United States, the dairy industry incurs annual losses of approximately US$200–250 million due to PTB (Ott et al. 1999). Comparable figures for the Brazilian beef sector are not well documented and warrant further investigation.

The spatiotemporal distribution of BPTB seroprevalence in Rio Grande do Sul from 2019 to 2023 reveals notable variability across regions and years. The highest seroprevalence over the study period was recorded in the Southeast mesoregion (0.52%), while no positive samples were detected in the Central-Eastern mesoregion. However, seropositive animals were identified in all other mesoregions. On an annual basis, the highest

seroprevalence classes were observed in the eastern and central mesoregions in 2019. In contrast, 2020 showed a marked reduction across most mesoregions. This decline may reflect shifts in the health status of animals selected for export during the COVID-19 pandemic, or selective culling of suspect animals at the farm level. A resurgence in 2022, particularly in the northern and eastern areas, may be linked to favorable environmental conditions for MAP survival (Whittington et al. 2004). The concentrated seroprevalence observed in the Southeast in 2023 may suggest regional differences in farm biosecurity practices or animal movement from previously affected zones (Weber et al. 2024). Meanwhile, the low seroprevalence observed in the northern mesoregions in 2023 may suggest effective local control measures, although this remains speculative without longitudinal farm-level data.

In our study, the overall seroprevalence of MAP in beef cattle herds across RS was low. This low detection rate may be partly explained by the limitations of ELISA-based diagnostics. Although ELISA is widely used in PTB surveillance due to its cost-effectiveness and scalability, its sensitivity varies by infection stage, often ranging from 25% to 50%, which may lead to underestimation of true prevalence (Collins et al. 2006). Furthermore, the extensive management systems common to beef cattle herds in RS are characterized by lower stocking densities and limited contact between age groups. This can potentially reduce MAP transmission risks when compared to intensive dairy systems (Whittington et al. 2019).

Most previous studies in Brazil have reported higher MAP seroprevalence than that observed in this study. Carrillo et al. (2019) detected antibodies against MAP in 97 of 1,800 (5.4%) export-bound cattle up to one year old from Goiás, Minas Gerais, and São Paulo. In Rio Grande do Sul, Setim et al. (2023a) found 20 seroreactive cows among 362 (5.5%) from dairy herds, whereas Setim et al. (2023b) found no seropositive beef cattle, with only 2 of 393 samples (0.5%) classified as suspect. These differences likely reflect variations in herd type, geographic origin, and management practices. The evaluated herds consisted of beef cattle, which are typically raised in extensive systems. In such systems, cattle have larger grazing areas per animal, reducing direct contact and, consequently, lowering or eliminating pathogen transmission and infection risk (Whittington et al. 2019).

Despite the low prevalence observed in this study, the detection of MAP-seropositive samples in nearly all mesoregions is notable, particularly considering that several of these herds participate in international trade. Countries such as Turkey require negative pre-export test results for paratuberculosis in live cattle, and even sporadic detections could threaten market access (MAPA 2021).

From a One Health perspective, PTB has implications beyond animal health. MAP has been detected in tissues of patients with Crohn's disease and other autoimmune conditions, raising concerns about a potential zoonotic link (Ekundayo and Okoh 2020; Dow and Alvarez 2022). While this association remains under debate, the survival of MAP through pasteurization and its presence in dairy and meat products underscore its relevance to food safety and public health (Gill et al. 2011; Albuquerque et al. 2019; Kuenstner and Kuenstner 2021). These findings highlight the importance of robust biosecurity measures and food safety protocols.

In addition to its role in enteric disease, MAP infection has been associated with secondary health problems in cattle, including mastitis and reduced reproductive performance (Scarpellini et al. 2023; Setim et al. 2023a). Infected animals may also be more susceptible to other diseases or conditions (Garcia and Shalloo 2015), which exacerbates economic and welfare concerns.

In conclusion, while MAP seroprevalence in beef cattle herds in RS remains low, its potential impact on public health, food safety, and international trade is considerable. Regional differences in seroprevalence also support the need for targeted control strategies, especially in mesoregions with higher detection rates. These findings underscore the necessity of sustained, standardized surveillance efforts to accurately track the epidemiology of paratuberculosis in endemic regions. Moreover, enhancing these efforts through the incorporation of molecular diagnostic tools could improve detection sensitivity. Finally, there is an urgent need for studies estimating the economic impact of PTB on the Brazilian beef sector to guide evidence-based policymaking and industry interventions.

Declarations

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

Approval from an animal ethics committee was not required for this study because only serum samples that had been collected for the official pre-export quarantine testing, and that would otherwise have been discarded, were used. No animal was handled or sampled for the purposes of this research.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

Conceptualization: Rodrigo Casquero Cunha; Investigation (sample collection and ELISA): Carolaine Garcia de Matos; Formal analysis: Carolaine Garcia de Matos, Oluwagbemiga Ademola Dada, Pedro Machado Medeiros de Albuquerque, Kauê Rodriguez Martins e Rodrigo Casquero Cunha; Writing – review and editing: Oluwagbemiga Ademola Dada, Pedro Machado Medeiros de Albuquerque, Kauê Rodriguez Martins e Rodrigo Casquero Cunha. All authors read and approved the final manuscript.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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Figures

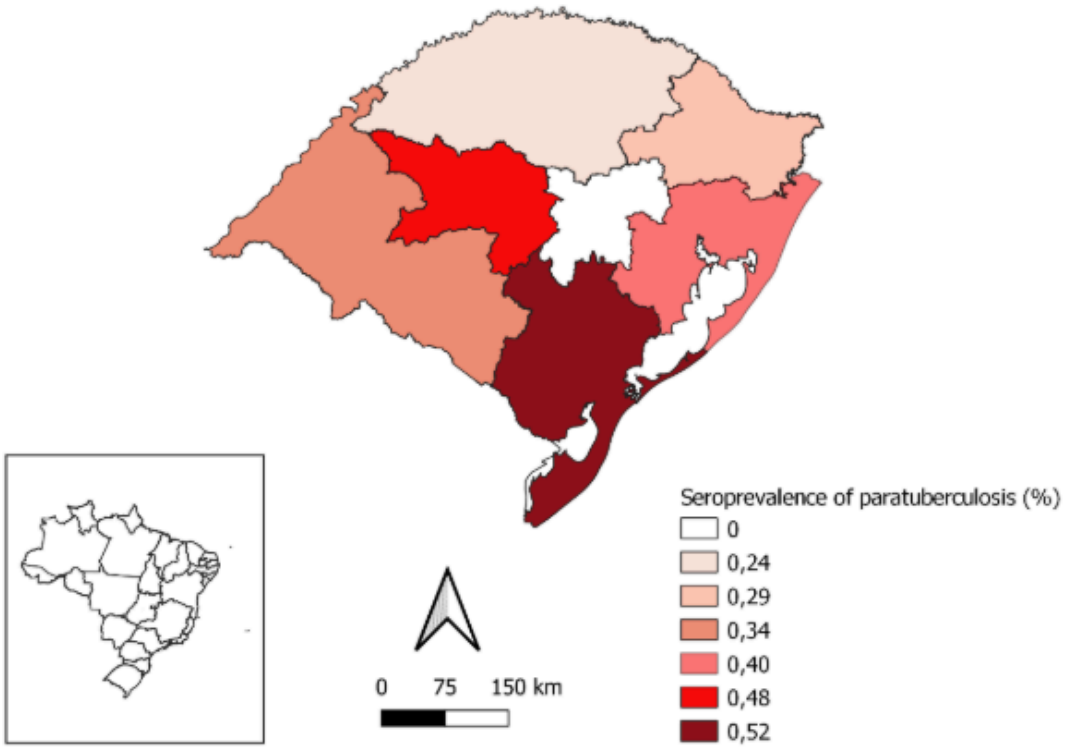


Figure 1

Categorized map showing the seroprevalence of bovine paratuberculosis by mesoregion in cattle quarantined for export in the state of Rio Grande do Sul, Brazil, over the years 2019, 2020, 2022, and 2023. Map generated in QGIS

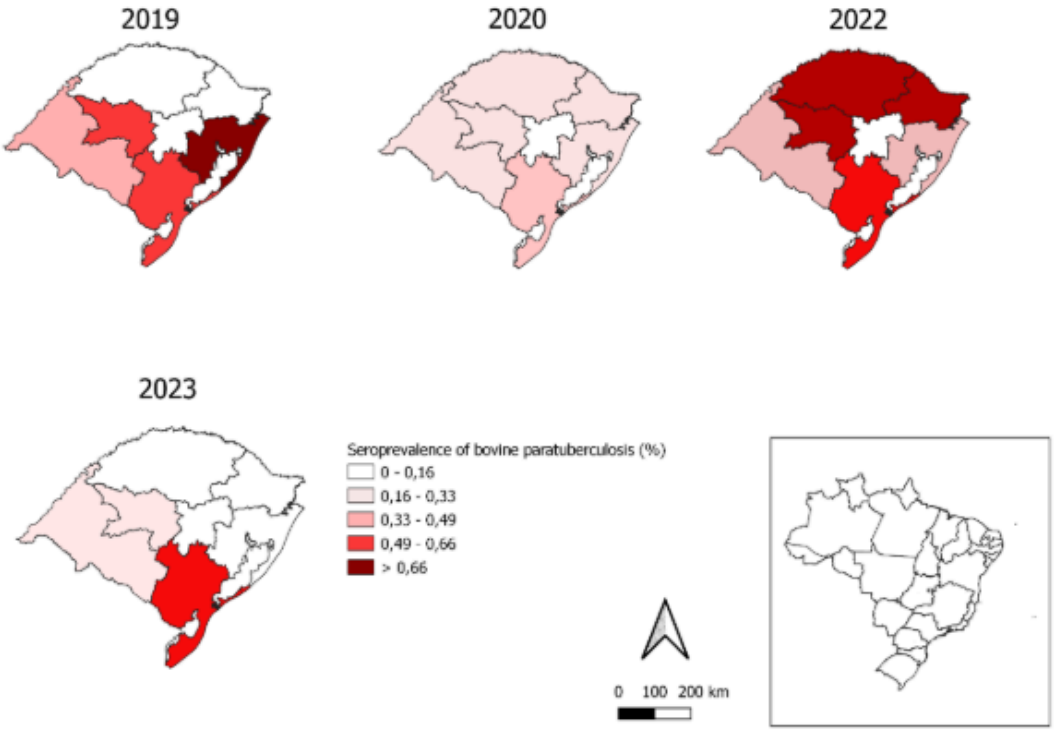


Figure 2

Graduated maps showing the annual seroprevalence of bovine paratuberculosis by mesoregion in cattle quarantined for export in the state of Rio Grande do Sul, Brazil, in 2019, 2020, 2022, and 2023. Maps generated in QGIS