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MICROBIOLOGY

Serological evidence of *Leptospira* spp. infection in livestock from indigenous villages in the Caatinga biome, Brazil

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ABSTRACT: Leptospirosis, a disease of significant economic impact on livestock and public health concern, may exhibit unique epidemiological characteristics in indigenous villages, particularly those located within semiarid climates such as the Caatinga biome in Brazil. This environment often presents conditions unfavorable for leptospire survival. This study conducted serological diagnoses of livestock in indigenous villages within the Caatinga biome, Northeastern Brazil. The survey was carried out in the Mãe Maria and Santa Helena indigenous villages, home to the Xukuru de Cimbres tribe, in the county of Pesqueira, Pernambuco State, Brazil, within the Caatinga biome. Blood samples were collected from cattle (n = 42), goats (n = 41), and sheep (n = 19). Out of the 102 samples tested, 73 (71.6%; 95% CI = 62.8% - 80.3%) were seroreactive (cut-off ≥ 100). The seropositivity rates for cattle, goats, and sheep were 54.8%, 80.5%, and 89.5%, respectively. A statistically significant difference was observed in the proportion of seropositive animals between cattle and goats and cattle and sheep ($P < 0.05$). Icterohaemorrhagiae was identified as the most probable serogroup across all animal species. The study revealed that livestock in indigenous villages within the Caatinga biome are frequently exposed to leptospires. This finding underscores the importance of a One Health approach, emphasizing the need for comprehensive studies in areas where large human and animal populations coexist. Such studies are crucial for investigating zoonotic infections and for planning and implementing control measures against livestock-associated leptospirosis.
Key words: leptospirosis, One Health, livestock, Caatinga biome, serology.

Evidência sorológica de infecção por *Leptospira* spp. em animais de produção de aldeias indígenas no bioma Caatinga, Brasil

RESUMO: A leptospirose causa perdas econômicas na pecuária e é um problema de saúde pública, sendo possível que existam peculiaridades na epidemiologia dessa doença em aldeias indígenas, principalmente naquelas de áreas de clima semiárido, como o bioma Caatinga no Nordeste do Brasil, onde o ambiente é frequentemente adverso à sobrevivência de leptospiras. Assim, o objetivo deste estudo foi realizar o diagnóstico sorológico em rebanhos de aldeias indígenas no bioma Caatinga. A pesquisa foi realizada nas aldeias indígenas Mãe Maria e Santa Helena, tribo Xukuru de Cimbres, município de Pesqueira, estado de Pernambuco, Brasil, dentro do bioma Caatinga. Amostras de sangue foram colhidas de bovinos (n = 42), caprinos (n = 41) e ovinos (n = 19). No geral, das 102 amostras testadas 73 (71,6%; IC 95% = 62,8% - 80,3%) foram sororreativas (ponto de corte ≥ 100). Para bovinos, caprinos e ovinos as taxas de soropositividade foram de 54,8%, 80,5% e 89,5%, respectivamente. Houve diferença estatística na proporção de animais soropositivos entre bovinos e caprinos e bovinos e ovinos ($P < 0,05$). Icterohaemorrhagiae foi o sorogrupo mais provável em todas as espécies animais. O estudo mostra que animais de produção de aldeias indígenas são comumente expostos a leptospiras no bioma Caatinga, o que constitui uma preocupação do ponto de vista de Saúde Única e demonstra a importância de estudos amplos em que coexistem grandes números de humanos e animais na investigação de infecções zoonóticas e no planejamento e implementação de medidas de controle da leptospirose associada a animais de produção.
Palavras-chave: leptospirose, Saúde Única, animais de produção, bioma Caatinga, sorologia.

Presently, incentives exist for the large-scale implementation of animal husbandry in indigenous villages, subtly incorporating a civilizational bias under discourses like bushmeat scarcity and food security (VELDEN, 2011). In fact, animal husbandry has been a significant component of indigenous culture in recent centuries, playing a crucial role in food autonomy by providing unrestricted access to its resources and in income generation (VELDEN, 2021).

Brazilian agribusiness is globally significant, particularly in terms of export dynamics. Livestock development has seen considerable growth in recent years across various regions of the country, including indigenous communities. However,

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these communities often remain on the periphery of agribusiness and are frequently overlooked in scientific studies. Reports of animal infectious diseases in these areas are scarce. Certain regional characteristics, such as climate, soil, vegetation, and topography, can contribute to the emergence of infectious diseases. One such disease is leptospirosis, which affects both domestic and wild animals as well as humans and has significant economic and public health implications (ELLIS, 2015). Leptospirosis, a zoonotic disease with a global distribution, is caused by bacteria from the *Leptospira* genus. These bacteria can survive for months in moist soils and water and can be accidentally transmitted to humans (KO et al., 2009). In endemic areas, livestock often shed leptospires without showing symptoms, highlighting the importance of this issue in terms of public health (VINCENT et al., 2019).

Brazil is home to approximately 7,000 indigenous villages (IBGE, 2019), yet there are no reports on the incidence of leptospirosis in livestock within these areas. The semiarid region of Brazil, characterized by low rainfall and high temperatures, along with the unique vegetation of the Caatinga—a biome exclusive to Northeastern Brazil with a rich wild fauna—presents distinct epidemiological conditions that necessitate evaluation in a context different from other regions in Brazil and globally. It is important to note that leptospirosis is a disease with a complex and dynamic epidemiology, involving intricate relationships between animals and a significant environmental component. As such, it should be studied from the One Health perspective. Consequently, this study conducted a serological diagnosis of leptospirosis in livestock from indigenous villages located in the Caatinga biome of Northeastern Brazil.

The survey was conducted in the indigenous villages of Mãe Maria and Santa Helena, which are part of the Xukuru de Cimbres tribe. These villages are situated in the municipality of Pesqueira, in the state of Pernambuco (Figure 1), within the Caatinga biome. This biome is unique to Brazil and is characterized by a semiarid climate, marked by prolonged periods of water scarcity and sparse vegetation. The climate is predominantly hot and dry, with a rainy season typically occurring in the summer and autumn months. Rainfall is primarily concentrated between March and April; although, precipitation can occur anytime from January through May. Droughts can persist for over a year, leading to a negative water balance and high solar radiation (BATISTA et al., 2007; ALVARES et al., 2014).

Currently, the tribe comprises roughly one thousand inhabitants, including both villagers and non-village residents. Their primary sources

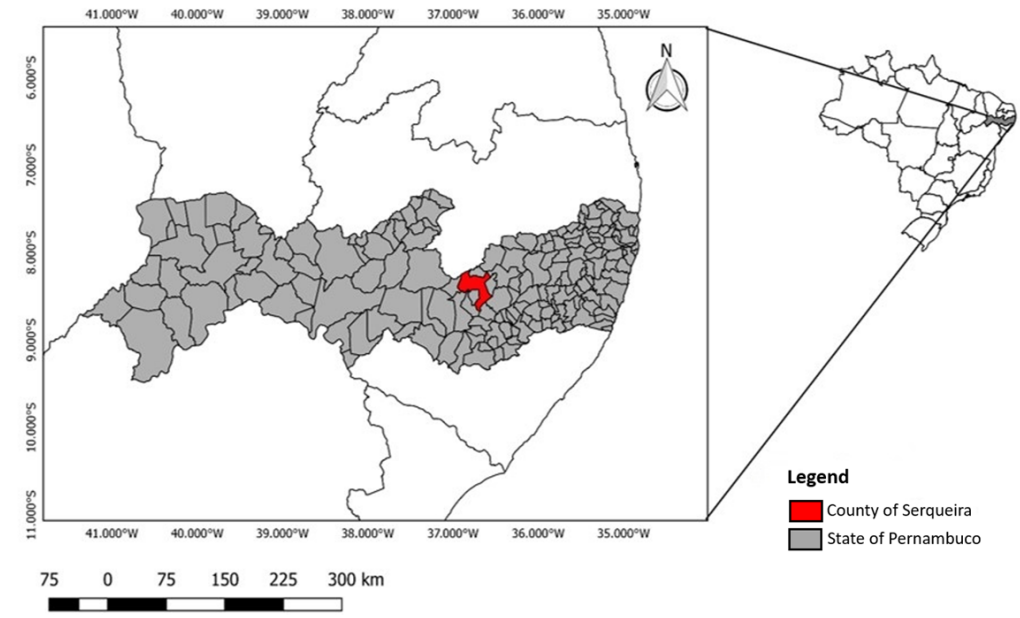

Figure 1 - Map with the geographic localization of the Pesqueira county, Pernambuco State. Grayed area on the map represents the Pernambuco state within Brazil.

of income are animal husbandry, agriculture, and handicraft production. The village's livestock primarily consists of dairy cattle, goats, sheep, and free-range chickens. The sale of these animals takes place at the city fair and in other nearby cities.

Owing to the scarcity of data on livestock populations in the indigenous villages of the Caatinga biome, a non-probabilistic (convenience) sampling method was employed. The Mãe Maria and Santa Helena tribes collectively had 283 cattle, 88 sheep, and 67 goats. In June 2021, among the Mãe Maria and Santa Helena tribes, blood samples were collected from 102 animals, including 42 cattle, 19 sheep, and 41 goats, after obtaining their owner's consent. These animals were raised on 10, three, and eight different properties, respectively. The blood samples were obtained by puncturing the jugular vein and collecting the blood in properly labeled 8 mL vacuum tubes. Following collection, the samples were maintained at room temperature until clot retraction and serum separation occurred. The serum was then stored in microtubes and frozen at -20 °C until the serology tests were conducted.

The detection of anti-*Leptospira* spp. antibodies was conducted using the microscopic agglutination test (MAT). This test utilized a collection of 24 serovars from 17 distinct pathogenic serogroups of five species, all of which originated from the Pasteur Institute in France. These included *L. interrogans* serovars Copenhageni (strain Wijinberg), Canicola (strain Hond Utrecht IV), Autumnalis (strain Akiyami A), Wolffi (strain 3705), Hardjoprajitno (strain Hardjoprajitno), Icterohaemorrhagiae (strain Verdun), Pomona (strain Pomona), Kennewicki (strain Fromm), Hebdomadis (strain Hebdomadis), Pyrogenes (strain Salinem), Bratislava (strain Jez Bratislava), and Australis (strain Ballico). The collection also included *L. santarosai* serovars Guaricura (strain BOV G), Shermani (strain 1342 K), and Canalzoni (strain CZ 188); *L. borgpetersenii* serovars Javanica (strain Poi), Tarassovi (strain Perepelitsin), Ballum (strain Mus 127), Mini (strain 2008/01925), and Castellonis (strain Castellon 3); *L. kirschneri* serovars Grippotyphosa (strain Moskva V) and Cynopteri (strain 3522 C); and *L. noguchi* serovars Panama (strain CZ 214 K) and Louisiana (strain Luc 1945) (OIE, 2018). Antibody titers were identified as the highest serum dilution yielding at least 50% agglutinated leptospires for each serogroup. Animals were considered positive if they exhibited titers ≥ 100.

The chi-square test or Fisher's exact test was used to compare seropositivity across different animal species, setting the significance level at 5% ($P \leq 0.05$). The Biostat 5.04 software was employed for data analysis.

In total, 73 out of the 102 tested samples (71.6%; 95% CI = 62.8%-80.3%) were seroreactive. The seropositivity rates for cattle, goats, and sheep were 54.8%, 80.5%, and 89.5% respectively (Table 1), with antibody titers ranging from 100 to 400. A statistically significant difference was observed in the proportion of seropositive animals between cattle and goats, as well as between cattle and sheep ($P < 0.05$). The detected serogroups were Icterohaemorrhagiae, accounting for 91.4% (64/70) of positive samples, and Sejroe, accounting for 8.6% (6/70) (Table 2). Icterohaemorrhagiae was the most prevalent serogroup across all animal species, while Sejroe was only detected in cattle. For three samples, the most frequent serogroup could not be determined owing to cross-reactions with identical antibody titers.

The observed frequency of positivity can be notably high, underscoring the potential for widespread infection among the studied species. Certain unique characteristics of indigenous tribes' animal rearing practices should be considered. These include geographic location, which provides access to forested areas and contact with wild and synanthropic animals (infection reservoirs), the presence of stray dogs within the community, the communal rearing of multiple animal species, and the use of extensive breeding methods. Other factors include the absence of

Table 1 - Cattle, goats, and sheep from the Xukuru de Cimbres indigenous tribe, Caatinga biome, Brazil, seroreactive to *Leptospira* spp. infection.

Animal species	Total number of animals	Number of seroreactive animals	Frequency (%)*
Cattle	42	23	54.8[a]
Goats	41	33	80.5[b]
Sheep	19	17	89.5[b]
Total	102	73	71.6

* Different lowercase letters within the same column signify a statistical difference between proportions ($P \leq 0.05$).

Table 2 - Reactive *Leptospira* spp. serogroups in cattle, goats, and sheep from the Xukuru de Cimbres indigenous tribe, Caatinga biome, Brazil.

Serogroup	----------Number of seroreactive animals (%)----------			Total (%)
	Cattle (%)	Goats (%)	Sheep (%)	
Icterohaemorrhagiae	15 (71.4)	33 (100)	16 (100)	64 (91.4)
Sejroe	6 (28.6)	-	-	6 (8.6)
Total (%)	21 (30)	33 (47.1)	16 (22.9)	70 (100)

technical assistance, a lack of disease-related information among owners, poor socioeconomic conditions, and a dearth of public service policies aimed to improve the community's sanitary infrastructure.

The animals sampled were sourced from indigenous villages, spanning an area exceeding one thousand hectares, of which over 800 hectares are preserved forest. These areas predominantly engage in extensive livestock rearing. The lack of health management for these animals, coupled with these environmental factors, may contribute to an increased frequency of leptospirosis. This community primarily consists of family farms, where most owners raise livestock for personal consumption and as a supplementary income source. Consequently, the rudimentary conditions under which these animals are raised should be considered. This is further compounded by the frequent absence of technical assistance for the herd, which is typically sought only in instances of significant economic losses on the properties.

Contrary to the general trend of leptospirosis prevalence being higher in cattle than in goats and sheep, the current study found statistically higher seropositivity rates in goats and sheep compared to cattle. Notably, sheep kept in semi-arid conditions have demonstrated resistance to *Leptospira* spp. and brief seroconversion periods (COSTA et al., 2017; COSTA et al., 2018). Additionally, studies have indicated that the MAT performs optimally with a cut-off point of 50, using PCR and bacteriological culturing as gold standards (NOGUEIRA et al., 2020). However, the potential existence of alternative transmission routes in sheep and goats kept in semi-arid conditions has been suggested, given the high PCR-positive rates in fluids and organs from the genital tract (NOGUEIRA et al., 2020; SOARES et al., 2021; SANTOS et al., 2022).

The Icterohaemorrhagiae serogroup was predominantly observed. The survey was carried out during the dry season when pastures are limited owing to low rainfall. The only available food source for the animals during this period is commercial feed stored in sheds, which can attract rodents. These rodents can contaminate the feed with their urine. The association between the presence of rodents and the incidence of leptospirosis in goats from the Brazilian semiarid region has been established (HIGINO et al., 2013). In sheep, the presence of the Icterohaemorrhagiae serogroup typically signifies accidental infection, often leading to acute leptospirosis syndrome outbreaks (MARTINS et al., 2012; GIANGASPERO et al., 2013). Moreover, serovars within this serogroup possess a high zoonotic potential. The Sejroe serogroup was also detected. It is commonly found in cattle worldwide, as they are considered adapted to this serogroup and serve as maintenance hosts (ELLIS, 2015).

Leptospires from the Icterohaemorrhagiae serogroup have been linked to severe clinical disease in humans (HAAKE & LEVETT, 2015; GRILLOVÁ et al., 2020). A survey in French Polynesia (GRILLOVÁ et al., 2020) collected blood samples from 15 patients who succumbed to leptospirosis. All patients were infected with strains from lineage 4, with 10 patients associated with the Icterohaemorrhagiae serogroup and five with the Australis serogroup. Given this situation, there is a pressing need for effective public health interventions to control livestock as potential sources of leptospires, which could cause fatal infections in the indigenous population.

In conclusion, the study reveals frequent exposure to leptospires among cattle, goats, and sheep in indigenous villages within the Caatinga biome. This finding underscores a One Health-based concern, highlighting the necessity for comprehensive studies in areas where humans and animals coexist. Such research is crucial when investigating zoonotic infections and in the planning and implementation of control measures for livestock-associated leptospirosis.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The Animal Ethics Committee (CEUA/CSTR/UFCG) granted approval for this study under protocol number 051/2019.

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