Communication

Anti-*Leptospira* antibodies in buffaloes in the Marajó Island

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Abstract: Leptospirosis is a zoonotic disease that has a cosmopolitan geographical distribution, reported in domestic and wild animals, which act as reservoirs and contribute to the spread of the microorganism in the environment. In Brazil, studies on the occurrence of leptospirosis in buffaloes in the Amazon biome are scarce. The objective of this study was to determine the occurrence of antibodies against *Leptospira* spp., including serovar Hardjo (Bolivia), isolated from cattle in Brazil and not yet tested in buffaloes. A total of 387 blood serum samples of animals from nine municipalities in the Marajó Island, state of Pará, northern Brazil, were obtained from a biological sample bank and analyzed using the serum agglutination microscopic test (SAM). Serology revealed 91.5% (387/354) of the animals tested positive for anti-*Leptospira* antibodies. Among the serovars tested, the serovar Hardjo (Bolivia) was the most prevalent, present in 79.3% of the reactive buffaloes.

Keywords: leptospirosis; diagnosis; *Bubalus bubalis*; zoonosis; Amazon Biome

1. Introduction

Leptospirosis is an infectious disease caused by the spirochete bacteria of the species *Leptospira*, which includes more than 260 reported serovars [1]. This zoonotic disease has a cosmopolitan geographical distribution, having been reported in domestic and wild animals, that act as reservoirs and contribute to the spread of the bacteria in the environment. The disease is more frequent in hot-humid tropical regions due to climatic characteristics such as abundant rainfall, heat and humidity, which favor the prevalence of the bacteria in the environment [2,3].

In Brazil, previous studies have identified the occurrence of anti-*Leptospira* antibodies in buffaloes [4,5,6,7,8], but in the seroepidemiological surveys, none used serovar Hardjo (Bolivia), isolated from cattle in Brazil and underused in buffaloes. Therefore, this is the first one's studies to evaluate the presence of this serovar in buffaloes.

The primary form of transmission of buffalo leptospirosis is the contact of susceptible animals with the urine of infected animals through the skin, mucous membranes and conjunctiva. In the Amazon region, buffaloes remain in flooded areas for long periods of time and may present subclinical conditions of the disease, which favors the epidemiological chain of the disease in the herd [9]. The main clinical signs of leptospirosis that can be observed are abortion and mastitis, which have direct negative impact on production, causing considerable losses to farmers and the livestock sector [7,10].

The number of buffaloes in Brazil is around 1.4 million head, and the state of Pará, in northern Brazil, is responsible for 38.2% of the total number of animals in the country, with Marajó Island accounting for the largest herd [11,12]. However, despite this considerable herd size, the production characteristics in the state, such as extensive grazing alongside other domestic species, and deficient hygienic-sanitary control, limit the productive potential of the buffalo sector [13]

Knowledge on the main *Leptospira* serovars circulating in the region is important for the adequate management of the disease and increase productivity, as are the isolation and treatment of diseased animals in addition to preventive vaccination in the herd [9,14]

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Given the importance of leptospirosis to the buffalo production chain, and the possible negative impacts of the disease in the livestock sector, the objective of this study was to determine the occurrence of antibodies against *Leptospira* spp., including serovar Hardjo (Bolivia) isolated from cattle in Brazil and underused in buffaloes.

2. Materials and Methods

The study was conducted using 387 samples of blood serum from Jafarabadi x Murrah crossbred buffaloes (*Bubalus bubalis*) of both sexes, with an average age of 36 months, obtained from the biological sample bank of the Veterinary Hospital of the Institute of Veterinary Medicine of the Federal University of Pará (Instituto de Medicina Veterinária da Universidade Federal do Pará – IMV-UFPA). The samples were collected over 12 months, comprising both the dry and wet seasons in the Amazon region. The sera used in this study were from animals from nine municipalities of Marjó Island, state of Pará: Cachoeira do Arari (n = 60), Santa Cruz do Arari (n = 60), Soure (n = 60), Salvaterra (n = 60), Chaves (n = 60), Melgaço (n = 16), Muaná (n = 3), Ponta de Pedras (n = 60) and Anajás (n = 8).

According to the history of the properties sampled, the buffalo herds were indigenous, were reared with low or no sanitary control, were only vaccinated for brucellosis and foot-and-mouth disease and were never vaccinated against leptospirosis. The buffaloes were reared in extensive grazing systems alongside other domestic species such as cattle, horses, pigs and small ruminants. All properties studied had a swath of land with native vegetation with presence wild animals, especially rodents that possibly are reservoirs bacteria *Leptospira* spp, and the buffaloes had access to this area. The animals submitted to blood collection had normal health status, without reporting by owners of abortion, mastitis or death near the time of blood collection.

The detection of anti-*Leptospira* spp. antibodies was performed in the Laboratory of Leptospirosis of the Veterinary School of the Federal University of Minas Gerais (Universidade Federal de Minas Gerais) using the microscopic agglutination test, which is considered the gold standard for leptospirosis diagnosis [15]. The battery of antigens used for the SAM comprised the serovars Bataviae, Bratislava, Hardjo type hardjo-bovis, Hardjo (CTG sample), Hardjo (OMS sample), Icterohaemorragiae, Pomona and Hardjo (Bolivia) (Table 1).

Table 1. Standards of *Leptospira* spp. per serogroup, serovar and strain used in the microscopic agglutination test (MAT) to detect anti-*Leptospira* antibodies in buffaloes in Marajó Island, Pará state (Brazilian Amazon). Data from Chiareli et al. [16].

Serogroup	Serovar	Strain
Bataviae	Bataviae	Swart
Australis	Bratislava	Jez Bratislava
Sejroe	Hardjobovis	Hardjobovis
Sejroe	Hardjo (CTG)	Hardjoprajitno (CTG)
Sejroe	Hardjo (OMS)	Hardjoprajitno (OMS)
Icterohaemorrhagiae	Icterohaemorrhagiae	Rga
Pomona	Pomona	Pomona
Sejroe	Hardjo (Bolivia)	Hardjobovis

The microscopic agglutination test (MAT) was the one of screening, considering positive animals with titration of 1:100. We considered animals with zero result or with one cross as non-reactive to the tested serovars, and animals with two to four crosses as seroreagent, according interpretation parameters by the World Organization for Animal Health [17].

3. Results

Of the 387 serum samples tested, 354 tested positive for the presence of anti-*Leptospira* spp. antibodies, indicating an occurrence rate of 91.5% (Table 2).

Table 2. Sample size (N) and frequency (%) of reactive and non-reactive animals in the detection of anti-*Leptospira* spp. antibodies in buffaloes from different municipalities in Marajó Island, the state of Pará (Brazilian Amazon) using microscopic agglutination test (MAT).

Municipality	Reactive	Non-reactive	Total

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	N	%	N	%	
Anajás	07	87.5	1	12.5	8
Chaves	58	96.6	2	3.4	60
Melgaço	14	87.5	2	12.5	16
Muaná	3	100	0	0	3
Ponta de Pedras	50	83.3	10	16.7	60
Salvaterra	56	93.3	4	6.7	60
Santa Cruz do Arari	50	83.3	10	16.7	60
Cachoeira do Arari	58	96.6	2	3.4	60
Soure	58	96.6	2	3.4	60
TOTAL	354	91.5	33	8.5	387

Serovar Hardjo (Bolivia) showed the highest reaction percentage, followed by the Hardjo (OMS sample) and Hardjo type hardjo-bovis serovars (Table 3). Other serovars that reacted in part of the animals analyzed were the Pomona and Hardjo (CTG sample). The serovars Bratislava, Icterohaemorrhagiae and Bataviae reacted less among the buffaloes tested.

Table 3. Number of animals (N) and frequency of seroactive animals for different serovars of *Leptospira* spp. tested in buffaloes from different municipalities in Marajó Island, the state of Pará (Brazilian Amazon) using serum agglutination microscopy (SAM).

Serovar	N	Frequency (%)
Bataviae	9	2.3
Bratislava	47	12.1
Hardjo type hardjo-bovis	248	64.1
Hardjo (CTG sample)	111	28.6
Hardjo (OMS sample)	251	64.8
Icterohaemorrhagiae	16	4.1
Pomona	179	46.2
Hardjo (Bolivia)	307	79.3

4. Discussion

We found a higher frequency of reactive buffalos than that reported in other Brazilian states (37.7% to 43.7% in São Paulo, 27.9% in Paraíba, 70.6% in Maranhão) and previously in Pará, with 80% [4-8]. The high frequency of animals testing positive in all municipalities studied, that evidence the environmental characteristics and the high incidence in the studied region, which has endemicity factors for leptospirosis [7]. Levels of precipitation are high in the Amazon region, with relative humidity tending to be above 80% and an average annual temperature of 26 °C [18]. These climatic traits favour the prevalence of the bacteria *Leptospira* spp in the environment and increase the transmission risk of leptospirosis, facilitating the entire epidemiological chain of the disease [19].

In addition to the region's geoclimatic characteristics, the buffalo production system adopted in the Amazon, in which buffaloes are reared extensively alongside other livestock species with no division within and between estate which makes it difficult to adopt unhygienic-sanitary management practices, reinforcing the hypothesis that leptospirosis spreads easily among Pará buffalo herds, including on Marajó Island, as previously reported by Barbosa [13]. The high frequency of leptospirosis observed may also be related to the contact of buffaloes with wild animals, whit rodents, from adjacent forest areas, which are possible sources and transmitters of the agent [3].

Buffaloes can also act as key epidemiological agents, serving as important sources and disseminators of leptospirosis due to their immersion behavior in water [20]. Water is considered a primary route of contamination for new hosts as it may contain bacteria [21]. As the Amazonian biome is mainly composed of rivers and lakes, it increases the risk of spreading leptospirosis between productive and wild animals, as well as humans [22]. Given the presence of rodents in the region and the contact of buffalo with contaminated urine, it is possible that buffalo is a link in the leptospirosis transmission chain for humans and other ruminants [23].

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This is the first study to report the infection of serovar Hardjo (Bolivia) in buffaloes in Marajó Island, Amazon Biome, Brazil. The high frequency of this serovar in our samples suggests the need to include serovar Hardjo (Bolivia) in the battery of diagnostic antigens for *Leptospira* spp. in buffaloes in Brazil, as animals are traded widely among the Amazon and other regions of Brazil. The high frequencies of other serovars of the Hardjo group in our samples suggests that these serovars, which have a known predilection for cattle, may also have a predilection for buffaloes. The combined rearing of cattle and buffaloes in our study sites may facilitate the interspecific transmission of serovars. Additional serological studies, as well as isolation and identification of bacterial agents, in buffaloes with the disease are needed to clarify the occurrence of Hardjo serovars in buffaloes in Brazil [24]

When compared to the data observed by Viana et al. [6] study conducted in the State of Pará, we can observe the occurrence of the Serovar Hardjo, they worked with of the serogroup Sejroe, considered more common, (CTG sample), Hardjo (WHO sample) and Hardjo (Bolivia), which are also from the Sejroe serogroup, with this we can suggest the need to use different serovars seroepidemiological research to obtain results with greater comprehensiveness. When more serovars are included in the surveys [2,19], the diagnosis becomes more specific and able to identify the most important serovars in the regions studied, optimizing the prophylactic and control measures to be adopted herds.

The prevalence of the Pomona serovar in our samples confirms the infection potential of this serovar in buffalo populations [25]. As buffaloes in Pará are kept in extensive grazing system in cohabitation with other livestock, the high frequency of the Pomona serovar in buffaloes might be related to cross-infection with pigs [3,5}. The same may be true for serovar Bratislava, due to contact of buffaloes with horses, the definitive host and source of infection by this specific serovar [26].

Serotypes Icterohaemorrhagiae and Bataviae were the least common among our samples, which can be explained by the extensive breeding system adopted in the farms where buffaloes were bred and with that the greater contact with the sources of infection with the serovars Icterohaemorrhagiae and Bataviae, like the rat and other animal reservoirs. These animal species are present in higher concentrations near the dwellings of properties and buildings storing feed, which serve as food sources [26-28], although the occurrence of rodents was reported at the farms sampled in this study. The occurrence of Icterohaemorrhagiae in ruminants was low, it was still, as well as other serovars reactive in the present study, are risk factors for the zoonotic occurrence of the disease, it is reiterated the importance of the use of different serovars in the battery of diagnostic tests of leptospirosis [16,29].

5. Conclusions

Anti-*Leptospira* antibodies were detected in buffaloes from nine municipalities of Marajó Island in Pará state, in the Amazon region of Brazil. The anti-serovar Hardjo (Bolivia) was the most prevalent. The presence and prevalence of various serovars detected may be related to the local practice of combined rearing of different livestock species, as well as to the contact with wild animals from adjacent forest areas, and rodents, all factors that likely facilitate the epidemiological chain of the disease in buffaloes. It is important to carry out serological surveys in order to identify the serovar that occurs in the herd with the objective of designing efficient strategies to control leptospirosis in the production of buffaloes.

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