

Prevalence of *Leptospira* serogroups in buffaloes from the Brazilian Amazon

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Abstract

Although Brazil has one of the largest buffalo populations in the Americas, buffalo leptospirosis is still poorly explored when compared to that in bovines; thus, the aim of this research was to carry out a large serological study for leptospirosis in this species in the Brazilian Amazon. For this, we collected 1,405 serum samples from buffaloes raised in the Amazon delta region, which is considered a major area of buffalo production in Brazil. The test used was a microscopic agglutination test (MAT) adopting 34 *Leptospira* antigens, some of which have never been tested for buffaloes in Brazil, including autochthonous strains; in total, 20 serogroups were evaluated. From the total of 1,405 serum samples, 894 (63.6%) reacted in the MAT to at least one of the 20 serogroups, and 511 (36.4%) did not react. The serogroups Sejroe, Autumnalis and Pomona were the most prevalent, with titres ranging from 100 to 12,800, and the autochthonous strains used were not significant in relation to the reference serovars. Leptospirosis in buffaloes seems to have a serological profile similar to leptospirosis in cattle, mainly due to the prevalence of the Sejroe serogroup; however, the results of this study suggested that in the Brazilian Amazon, *Leptospira* strains that are serologically distinct from the autochthonous strains isolated in the southeastern region of Brazil may be circulating in these animals. Other serovars could also be inserted into the panel of antigens used in MAT for serological studies on buffaloes.

KEYWORDS

Amazonia, Brazil, Buffalo, *Leptospira*, MAT

1 | INTRODUCTION

Buffaloes are considered tolerant animals and can be used for various types of livestock production; they have been prospected as possible alternatives for food production and agriculture in less

developed regions of the world (Desta, 2012). This trend has occurred specifically because buffaloes perform better than cattle in reproductive parameters and have the ability to convert low quality food (Bernardes, 2007), even in flooded areas such as in the floodplain of the Amazon River, where buffalo productivity is greater

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than that from raising bovines (Sheikh, Merry, & McGrath, 2006). Although these advantages are attributed to buffaloes, there are still many gaps in knowledge regarding the genetics, zootechnical characteristics and rusticity of these animals (El Debaky et al., 2019).

By 2,000, the buffalo population in Brazil had become the most crescent herd of the world (Vale, Minervino, Neves, Morini, & Coelho, 2013), and in recent years, it has come to have an effective population of approximately 950,000 head. It should be highlighted that approximately 50% of these animals are concentrated in the northern region of the country, more specifically in the area known as Amazon delta, which comprises part of the Amapá and Pará states, including Marajó Island (IBGE & Instituto Brasileiro de Geografia e Estatística, 2017). The activity has become so important in the region that in Amapá, there more buffalo that are slaughtered than cattle (Soares, Esteves, Fariam, Texeira, & Araujos, 2014).

Leptospirosis appears to manifest in buffaloes in the same way as in cattle, with special emphasis on reproductive disorders such as abortion (Balakrishnan, Meenambigai, & Roy, 2014; Marianelli et al., 2007), but there have also been reports of jaundice (Upadhye, Rajasekhar, Ahmed, & Krishnappa, 1983) and mastitis (Ahmed, 1990). The isolation of *Leptospira* in urine from a healthy buffalo (Vasconcellos et al., 2001) as well as the detection through PCR of bacteria in the urine of asymptomatic buffaloes show that these animals can also become reservoirs of leptospires in the environment by elimination through the urine (Denipitiya, Chandrasekharan, Abeyewickreme, Hartskeerl, & Hapugoda, 2017).

The serological studies conducted in Brazil for buffaloes involve various sample sizes and number of serovars are used as antigens in the panel of the microscopic agglutination test (MAT), which is generally the same standard used for most other animal species. A study carried out in São Paulo state that used 24 antigens in the MAT panel revealed a prevalence that 43.7% of buffaloes were reactive in a total of 879 animals examined (Favero et al., 2002); in Vale do Ribeira, São Paulo state, 37.7% of positive results were found in 403 animals assessed for only 10 antigens with the MAT (Langoni, Fava, Cabral, Silva, & Chagas, 1999); in Vale do Ribeira, another study of 222 buffaloes showed a that 50.9% of animals were tested positive with the use of 24 antigens in the MAT (Fujii, Kasai, Vasconcellos, Richtzenhain, & Cortez, 2001). In the Amazon region, a study verified a prevalence of 34.37% in 256 animals examined for 25 antigens with the MAT (Oliveira, Silva, Pinheiro, & Langoni, 2013), and in 212 samples of buffaloes examined in Pará state, 80.0% of the animals were reactive in a MAT with a panel of 27 antigens (Viana et al., 2009).

The main purpose of this study was to add knowledge to buffalo leptospirosis in Brazil through a serological study conducted in the Brazilian Amazon using an expanded panel of antigens in MAT, including some reference serovars and autochthonous strains isolated in Brazil never tested before for buffaloes.

2 | MATERIALS AND METHODS

This work used the region known as the Amazon delta as the study area, where the great Amazon River flows into the Atlantic Ocean in extreme northern Brazil, between the states of Pará and Amapá (Figure 1). This region has a humid equatorial climate characterized by high temperatures (average of 26°C) and high rainfall throughout the year (2,300 mm) as well as tropical vegetation that shelters a great diversity of plant and animal species (Fisch, Marengo, & Nobre, 1998). One unique aspect of this region is the large flood areas with daily variations in the river water level (Vogt et al., 2016), which favours the buffalo production in extensive systems, especially for meat production (Soares et al., 2014).

In January 2019, we collected blood from 1,405 buffaloes slaughtered at a slaughterhouse located in the Macapá microregion, Amapá state, which receives animals from different farms in the Amazon delta. The sampling was by convenience, and the animals were predominantly from the river buffalo group (the Murrah, Mediterranean and Jafarabadi breeds), composed by males and females of at least 12 months of age that had the ability to produce meat and that lacked records of vaccination against leptospirosis in addition to unknown reproductive situations.

The microscopic agglutination test (MAT) was performed following Faine, Adler, Bolin, and Perolat (1999) and employed a panel of 34 live antigens that represented 20 different serogroups; these antigens were reference serovars and autochthonous strains isolated in Brazil (Table 1).

Statistical analysis was carried out through descriptive statistics using frequency measures. For general prevalence, all reactive samples were considered at a 1:100 dilution, and for determination of the most prevalent serogroups, the ranking technique was used, which considered the only reactive samples for the antigen that had the highest titre; animals that were reactive to more than one antigen with a predominant titre were disregarded for this analysis (Vasconcellos et al., 1997).

3 | RESULTS

Of all 1,405 serum samples, 894 (63.6%) reacted in the MAT to at least one of the 20 serogroups, and 511 (36.4%) did not react. Considering only the reactive samples by ranking technique (665/894), the titres ranged from 100 to 12,800 (Table 2); the serogroups Sejroe, Autumnalis and Pomona were most prevalent, whereas no reactions were detected for the serogroups Bataviae, Canicola, Celledoni, Javanica, Pyrogenes, Mini and Shermani.

The Sejroe serogroup had the highest number of serovars tested, revealing a very different reaction profile among the serovars. Ricardi and Medanensis were the most prevalent inside the Sejroe serogroup (Figure 2). The serovars Hardjo-prajitno, Wolffii and Guaricura, which are widely used as representatives of this serogroup, had reaction percentage lower than 5.0%.



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FIGURE 1 Geographical location of the Amazon delta in Brazil

Among the autochthonous strains isolated in Brazil that were used in this study, serovar Ranarum had the highest number of reactions, while the others were not expressive (Figure 3). All autochthonous serovars used were isolated from animals in the southeastern region of Brazil as there have thus far been no isolates from the Brazilian Amazon.

4 | DISCUSSION

Leptospirosis in buffaloes has been reported in several countries through serological studies that indicate different prevalences, such as 14.6% (33/226) in buffalo raised in Trinidad, Caribbean (Adesiyun, Hull-Jackson, Clarke, Whittington, & Seepersadsingh, 2009); 22.2% (111/500) in Argentina (Konrad et al., 2013); 25% (3/12) in Egypt (Felt et al., 2011); 48% (81/170) in the Philippines (Villanueva et al., 2016); 58.73% (111/189) in Iran (Hajikolaei, Ghorbanpour, & Abdollahpour, 2006); and 88.8% (111/125) in India (Selvaraj et al., 2010). In Thailand, a high prevalence of leptospirosis in buffaloes has been reported (Tangkanakul, Smits, Jatanasen, & Ashford, 2005; Wongpanit, Suwanacharoen, & Srikrum, 2012), which has become a public health concern in some regions of Asia, such as Sri Lanka, where a large part of the economy is based on agriculture that relies on rice cultivation in flooded fields and uses buffaloes in their activities, implying a possible relationship of these animals with human leptospirosis in the country (Agampodi et al., 2011). The use of an expanded antigen panel in the MAT may have increased

the sensitivity of the test for this work, but the prevalence found in this study (63.6%) showed that leptospirosis was present in the buffaloes of the Brazilian Amazon. This still deserves attention from a public health perspective, since in the Amazonia, it is common to use these animals for food through the consumption of meat and milk. In addition, buffaloes are part of the local culture and are also used in both labour and tourism, causing closer contact with humans (Barreto, Lobato, Pereira, & Serra, 2017). A limitation that occurred in this study was the logistical conditions at the slaughterhouse, because during the slaughter of the animals it was not possible to realize an association between the characteristics of each animal and epidemiological information with it is respective serum sample.

Water is one of the major source of leptospirosis contamination (Faine et al., 1999), and this could reflect one of the risk factors for buffaloes, since in addition to their gregarious habits, they tend to seek out water for wallowing and bathing, mainly in hot weather, with the objectives of thermoregulation and protection from ecto-parasites (Napolitano, Pacelli, Grasso, Braghieri, & Rosa, 2013). In the Amazon, it is also common to observe buffaloes in rivers and dammed areas along with wild animals, mainly those of semi-aquatic habits, such as capybaras (*Hydrochaeris hydrochaeris*), which have already been reported to be able to eliminate leptospires through urine (Marvulo et al., 2009).

The Sejroe serogroup was the most prevalent in the animals, which indicated a similarity with bovine leptospirosis in Brazil, since this serogroup was the most prevalent (Sarmiento et al., 2012; Pinna et al., 2018), especially serovar Hardjo, which has been isolated

TABLE 1 Serovars of *Leptospira* spp. used as antigens in the microscopic agglutination test (MAT) listed by serogroups

Serogroup	Serovar
Australis	Australis
	Bratislava
Autumnalis	Autumnalis
	Butembo
Ballum	Castellonis
Bataviae	Brasiliensis ^{a,b}
Canicola	Canicola
Celledoni	Whitcombi
Cynopteri	Cynopteri
Djasiman	Sentot
Grippotyphosa	Grippotyphosa
	Banana ^{a,c}
Hebdomadis	Hebdomadis
Icterohaemorrhagiae	Copenhageni
	Icterohaemorrhagiae
Javanica	Javanica
Mini	Mini
Panama	Panama
Pomona	Pomona
	Pomona (GR6) ^{a,d}
Pyrogenes	Pyrogenes
Ranarum	Ranarum ^{a,e}
Sejroe	Gorgas
	Guaricura ^{a,f}
	Hardjo-prajitno
	Hardjo-bovis
	Medanensis
	Polonica
	Recreo
	Ricardi
	Sejroe
	Wolffi
Shermani	Shermani
Tarassovi	Tarassovi

^aAutochthonous strains isolated in Brazil.^bSanta Rosa, Sulzer, and Pestana de Castro (1972).^cMarvulo et al. (2002).^dMiraglia et al. (2008).^eYasuda et al. (1986).^fVasconcellos et al. (2001).

in Brazil from cases of leptospirosis in cattle (Chiareli et al., 2012; Chideroli et al., 2016). In buffaloes, the serovar Hardjo also stands out within the Sejroe serogroup (Favero et al., 2002; Rocha, Lima, Paz, Langoni, & Moraes, 2019; Viana et al., 2009), disagreeing with the results of the present study, where the serovars Ricardi and Medanensis were the antigens responsible for the prevalence of the

Sejroe serogroup over the others, since 33.5% of the samples were reactive only for the serovars Ricardi and/ or Medanensis, against 5.0% of the samples reactive only for the serovars Hardjo-prajitno, Hardjo-bovis, Guaricura and/ or Wolffi (data not shown).

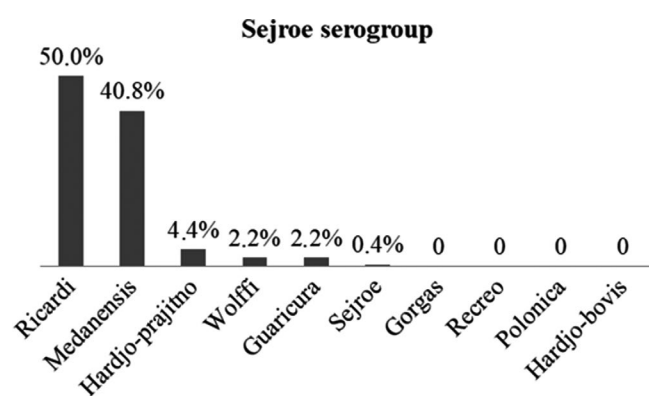
The first report of serovar Ricardi isolation occurred in a patient in Malaysia (Alexander, Evans, Toussaint, Marchwicki, & McCrumb, 1957), and it was subsequently isolated from intraocular samples of horses with recurrent uveitis (Hartskeerl et al., 2004), which suggested that this serovar could be circulating in animals. Serovar Medanensis was first described in Indonesia, where it was isolated from a healthy dog (Kouwenarr & Wolff, 1929) and has rarely been reported in the literature; consequently, serological reactions occurring for medanensis are considered cross-reactions with serovar Hardjo (Loewenstein, McLachlan-Troup, Hartley, & English, 2008). These serovars belong to two distinct subgroups within the Sejroe serogroup. Serovar Ricardi is inserted in subgroup Saxkoebing, and serovar Medanensis was introduced into subgroup Wolffi along with serovars Hardjo, Gorgas, Wolffi, Recreo and Guaricura, among others. There exists a third subgroup, called Sejroe, which includes the serovars Sejroe and Polonica (Kmety, 1977). Neither serovars has ever been used in serological studies in Brazil to represent the Sejroe serogroup.

In Brazil, it was agreed to use the strains of serovars Hardjo and serovar Wolffi in the MAT panel to represent the Sejroe serogroup; however, if only these serovars were used in this work, the Sejroe serogroup would not have been the most prevalent because few reactions were detected for the cited antigens. In the same way, serovar Guaricura, strain M4/98, which is isolated from the urine of a healthy buffalo in São Paulo (Vasconcellos et al., 2001), was also included in panel of antigens used in the MAT after having been observed as one of the most prevalent serovars in cattle in the central-western and southeastern regions of Brazil (Sarmiento et al., 2012); however, this antigen no longer seemed to be representative for animals in other areas of the country, such as buffaloes in northeastern Brazil (Oliveira et al., 2018) and in cattle in the Amazon (Guedes et al., 2019). This evidence reinforced the hypothesis of regional variability in the strains, influenced by climatic and environmental issues (Plank & Dean, 2000), especially in a country as extensive and heterogeneous as Brazil. The inclusion of serovars Ricardi and Medanensis into the panel of antigens used in the MAT to perform serological studies on buffaloes should be evaluated, particularly with the aim of increasing reactions to the Sejroe serogroup. In addition, although cross-reactions among serovars of the same serogroup happen in serology tests (Faine et al., 1999), the possibility that these two serovars are circulating in the buffaloes of the Amazon region cannot be excluded, therefore, further studies should also be encouraged to evaluate this condition, mainly aimed at the isolation of leptospires from these animals.

The Autumnalis serogroup was the second most prevalent; in a similar manner to the trend seen in the Sejroe serogroup, this trend seemed to be influenced by the antigens that represented it in MAT. In this study, serovar Butembo was involved in 98.7% of reactions within the Autumnalis serogroup (data not shown). In other countries, the Autumnalis serogroup was not expressive at serology for

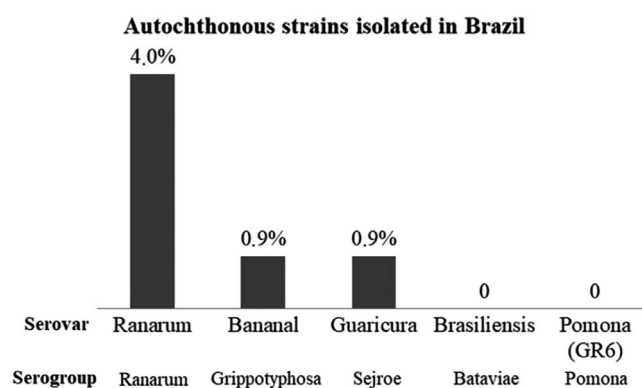
TABLE 2 Frequency of antibody titres found for 13 serogroups of *Leptospira* spp. in relation to the number of reactive samples by the ranking technique

Serogroups	Titres								Total	Total (%)
	100	200	400	800	1,600	3,200	6,400	12,800		
Sejroe	49	99	96	26	4	—	—	—	274	41.2
Autumnalis	43	71	36	3	—	—	—	—	153	23.0
Pomona	15	40	25	20	4	4	—	1	109	16.3
Grippotyphosa	7	15	11	4	3	—	—	—	40	6.0
Tarassovi	6	12	4	4	1	—	1	—	28	4.2
Ranarum	10	14	3	—	—	—	—	—	27	4.1
Sentot	11	1	—	—	—	—	—	—	12	1.8
Hebdomadis	5	3	—	—	—	—	—	—	8	1.2
Panama	1	3	—	—	—	—	—	—	4	0.6
Ballum	—	1	1	1	—	—	—	—	3	0.5
Icterohaemorrhagiae	—	2	1	—	—	—	—	—	3	0.5
Cynopteri	2	—	1	—	—	—	—	—	3	0.5
Australis	—	1	—	—	—	—	—	—	1	0.1

**FIGURE 2** Percentage of reactive samples for each serovar representative of the Sejroe serogroup used in relation to number of reactive for this serogroup

buffalo leptospirosis, as antigens different other than those of the Butembo serovar were used to represent this serogroup (Adesiyun et al., 2009; Balakrishnan, Padian, Roy, & Chandran, 2015; Konrad et al., 2013). In Brazil, serovar Butembo appears to be related to rodents and wild animals (Lilenbaum, 1996) and is widely used in serology; in cattle, it has been reported as one of the most prevalent serovars (Tonin et al., 2010) and is also associated with reproductive disorders in animals (Saldanha et al., 2007).

The Pomona serogroup followed the most prevalent serogroups, as described in buffaloes in the northeastern region of Brazil, making this serogroup one of the most dominant (Pimenta et al., 2019). In the present work, the autochthonous strain GR6 (Pomona), which was isolated from a slaughtered pig in São Paulo (Miraglia et al., 2008), did not generate reactions detected in MAT for serogroup Pomona, and all reactions for these serogroups were derived from the reference Pomona antigen, in contrast with what has

**FIGURE 3** Percentage of reactive samples for each autochthonous strain used in relation to total number of reactive samples by the ranking technique

already been reported in cattle in Amazonia, where all reactions detected for the Pomona serogroup were obtained by the GR6 strain (Guedes et al., 2019).

Among all five of the autochthonous strains isolated from animals in Brazil that were used in this study, the Ranarum sample obtained the largest number of reactions. This strain was isolated from the kidney of an aborted equine fetus and was characterized serologically as belonging to the saprophytic species of *Leptospira*, but it was not possible to conclude whether this was the cause of the abortion (Yasuda, Sulzer, Giorgi, & Soares, 1986). The use of this antigen had the aim of evaluating the behaviour of the Ranarum serogroup in buffalo leptospirosis in Brazil, since it shares saprophytic and pathogenic strains and had not yet been tested in the country for this animal species; in Thailand, serovar Ranarum was part of the panel of antigens used in MAT and was found to be one of the most prevalent in buffaloes (Chadsuthi et al., 2017). In cattle

in Malaysia, it surpassed even the Sejroe serogroup (Suwancharoen, Chaisakdanugull, Thanapongtharm, & Yoshida, 2013).

The introduction of locally isolated strains into the MAT panel has been reported as a way to increase the sensitivity of this technique to detect leptospirosis-positive animals (Sarmiento et al., 2012); in some cases, this method results in more reactions for locally isolated strains than for the reference strains, that end up being standardized on the MAT from region to region (Daud et al., 2018). Diverging from this trend, the present study found that autochthonous strains isolated in southeastern Brazil were not representative of the serology regarding the increase in reactions in general prevalence, as was observed in a serological study for cattle carried out in the Brazilian Pantanal, where nine strains isolated from different regions of Brazil were used and reacted at very low percentages (Miashiro et al., 2018).

In conclusion, this study showed that the Sejroe serogroup was the most prevalent in buffaloes, as in cattle; however, serovars Ricardi and Medanensis were the most prevalent in this serogroup, surpassing the Hardjo and Wolffi antigens traditionally used in Brazil, and could be included in the MAT panel to evaluate the true participation of these antigens as representatives of the Sejroe serogroup. In addition, the autochthonous strains isolated in the southeastern region of Brazil that were used in this study were not relevant, promoting the assumption that other local and serologically distinct strains may be circulating in buffaloes of the Amazon region.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

Israel Barbosa Guedes: Conceptualization; Investigation; Methodology; Writing-review & editing. Gisele Oliveira de Souza: Methodology. Lilian Abigail Ribeiro de Oliveira: Methodology. Juliana Fernandes de Paula Castro: Methodology. Antonio Souza Filho: Methodology. Anderson Luiz Pinheiro Maia: Methodology. Marcos Bryan Heinemann: Conceptualization; Investigation; Project administration; Validation; Writing-review & editing.

ETHICAL APPROVAL

This work was approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science (Universidade de São Paulo) – CEUA/FMVZ nº 5,613,211,118.

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