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Serological frequency of *Leptospira* spp. in buffaloes (*Bubalus bubalis*) in Paraná state, Brazil¹

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ABSTRACT.- Krueger L., Biondo A.W., Kmetiuk L.B., Lara M.C.C.S.H., Castro V., Dib C.C., Oliveira R.A.M., Perotta J.H. & Barros Filho I.R. 2023. **Serological frequency of** *Leptospira* **spp. in buffaloes** (*Bubalus bubalis*) **in Paraná state**, **Brazil**. *Pesquisa Veterinária Brasileira* 43:e07147, 2023. Universidade Federal do Paraná, Setor de Ciências Agrárias, Rua dos Funcionários 1540, Curitiba, PR 80035-050, Brazil. E-mail: louisekrueger@hotmail.com

Leptospirosis is a zoonosis that can infect buffaloes and other mammalians, and it's caused by Gram-negative bacteria of the genus *Leptospira* spp. The infection occurs through contact with urine or a contaminated environment with the mucous membranes of the animals, and the disease has two phases: leptospiremia and leptospiruria. The objective of this study was to determine the frequency of leptospirosis in buffaloes in the state of Paraná, Brazil, as well as the main serovars present in this area. A total of 276 blood samples were collected from buffaloes from 18 properties located in different cities and mesoregions of Paraná state. A microscopic agglutination test (MAT) was performed to detect the presence of anti-*Leptospira* spp. antibodies and to identify its serovars. The prevalence was 77.17% (213/276) of buffaloes seropositive for at least one serovar and serovar Hardjo. This study seems to be the first to determine the frequency of buffaloes with leptospirosis in the state of Paraná. Prevention and control measures are important to control the disease in herds.

INDEX TERMS: Leptospirosis, buffaloes, *Bubalus bubalis, Leptospira*, seroepidemiology, Icterohaemorrhagiae, Hardjo, Pomona, Brazil.

RESUMO.- [Frequência sorológica de *Leptospira* spp. em búfalos (*Bubalus bubalis*) no estado do Paraná, Brasil.] Leptospirose é uma zoonose que infecta búfalos e outros mamíferos e é causada por bactérias Gram-negativas do gênero *Leptospira* spp. A infecção ocorre por meio do contato com a urina ou ambiente contaminado com as membranas mucosas dos animais e a doença possui duas fases: leptospiremia e leptospirúria. O objetivo deste trabalho foi determinar a frequência da leptospirose em bubalinos provenientes do estado do Paraná, assim como determinar os principais sorovares presentes no estado. Um total de 276 amostras de sangue foram coletados de búfalos provenientes de 18 propriedades localizadas em 14 municípios de diferentes mesorregiões do estado do Paraná. O teste de soroaglutinação microscópica (SAM) foi realizado para detecção da presença de anticorpos anti-*Leptospira* spp. e identificação dos respectivos sorovares. A prevalência de búfalos soropositivos para ao menos um sorovar foi de 77,17% (213/276). O sorovar mais frequente identificado nestes búfalos foi o Icterohaemorrhagiae, seguido pelos sorovares Pomona e Hardjo. Este é o primeiro estudo a determinar a frequência de búfalos com leptospirose no estado do Paraná. Medidas de controle e prevenção são importantes para o controle do agente nos rebanhos.

TERMOS DE INDEXAÇÃO: Leptospirose, búfalos, *Bubalus bubalis, Leptospira*, soroepidemiologia, Icetrohaemorrhagiae, Hardjo, Pomona, Brasil.

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INTRODUCTION

Buffaloes are animals that easily adapt to different geoclimatic conditions and are known for their rusticity (Viana et al. 2009, Guedes et al. 2020a). However, knowledge about diseases that negatively affect their production, reproduction and factors that directly influence the economic viability of the production of these animals becomes necessary (Viana et al. 2009, Contreras et al. 2012). Among the infectious and contagious diseases that affect the health, production and reproduction of buffaloes' leptospirosis can be highlighted, which is also important from a public health aspect (Chadsuthi et al. 2018).

Leptospirosis is a zoonosis caused by gram-negative bacteria of the genus *Leptospira* spp. belonging to the family Leptospiraceae, of the order Spirochetales (Adler & de la Peña 2010, Oliveira et al. 2013, Pimenta et al. 2019). The bacteria have several pathogenic serovars, whose distribution varies according to region, climate and susceptible hosts (Levett 2004, Balamurugan et al. 2016). Leptospira proliferates mainly in tropical and subtropical climate regions, where they survive long periods in hot, rainy and humid environments (Miller et al. 1991, Saito et al. 2013). These microorganisms can remain in mud, swamps, lakes and vegetation (Hassanpour & Mousavi 2007, Wasiński & Dutkiewicz 2013), places where buffaloes have the habit of bathing, favoring the spread of leptospirosis in their herds (Nardi Júnior et al. 2011), since it facilitates the consumption and contact of the ocular, nasal, oral or genital mucous membranes with water contaminated by *Leptospira* spp. (Hajikolaei et al. 2006, Giraldo et al. 2014).

Most leptospirosis cases in buffaloes are asymptomatic, characterized mainly by reproductive problems such as repeated oestrus and abortions in the final third of pregnancy (Galiero 2007, Marianelli et al. 2007, Ijaz et al. 2020). In acute cases, the disease buffaloes may present clinical signs such as lethargy, anorexia, fever, hemorrhages, jaundice, agalactia, mastitis, diarrhea, and abortions (Galiero 2007, Felt et al. 2011, Oliveira et al. 2013). Buffaloes can also be pathogen reservoirs since they may be asymptomatic and transmit the disease to humans and other mammals (Felt et al. 2011, Denipitiya et al. 2017).

However, no studies have been found in the literature determining the prevalence of leptospirosis in buffaloes in the state of Paraná, Brazil. Thus, the objective of the present study was to perform the prevalence and detection of the main serovars of leptospirosis in buffaloes from the state of Paraná.

MATERIALS AND METHODS

Blood samples were taken from 276 healthy buffaloes from 18 herds in the state of Paraná. The inclusion criteria were not having a history of vaccination against leptospirosis and being buffaloes raised in rural properties of Paraná. It was stipulated to use about 10% of the animals from each property randomly chosen for blood sampling. The animals used in the study came from different properties in different cities of the state of Paraná, as shown in Figure 1.

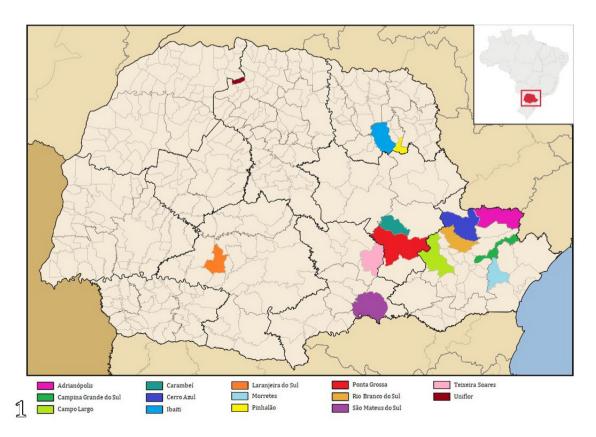


Fig.1. Map of the state of Paraná, southern Brazil, demonstrating the cities where the buffaloes' blood samples were collected.

Approximately 20mL of blood was collected from each animal through jugular or coccygeal venipuncture after adequate restraint of the animals. Previously identified 10mL collection tubes without anticoagulant with clot activator gel (BD, Vacutainer®) were used to collect the material. The blood samples were kept refrigerated in isothermal boxes until blood clot formation and then centrifuged at 600xG for 10 minutes for subsequent separation and collection of serum from each sample. The material was stored in microtubes of 1.5mL capacity properly identified and frozen at -80°C until it was sent to the laboratory.

The blood serum samples were sent to the laboratory of bacterial diseases of reproduction of the "Instituto Biológico de São Paulo" in the city of São Paulo/SP, Brazil. The diagnostic test performed was the MAT for antibodies detection for leptospirosis and identification of the following serovars: Icterohaemorrhagiae, Canicola, Pomona, Grippotyphosa, Hardjo, Bataviae, Autumnalis, Bratislava, Pyrogenes, Shermani, Sentot, Guaricura, Whitcombi, Andamana and Patoc. Reactive samples were considered those with minimum dilution titers of 1:100.

Statistical analysis was performed descriptively using frequency measures. The odds ratio (OR) test was performed to check the association between being seropositive to *Leptospira* spp. and exposure or not to some risk factors and reports of reproductive problems. Significant differences were obtained using the chi-square test, and the tests were considered significant when p<0.05.

This project was approved by the ethics committee on the use of animals from the "Setor de Ciências Agrárias" (Agricultural Sciences Sector) of the "Universidade Federal do Paraná" (UFPR) under protocol 043/2020.

RESULTS

Of the 276 samples collected from apparently healthy buffaloes, 77.17% (213/276) were positive for at least one serovar of *Leptospira* spp., with titers ranging from 1:100 to 1:800 in the MAT test. Table 1 shows the prevalence for the respective serovars; only the serovars with at least one serovar reagent are listed.

The serovars Icterohaemorrhagiae (92.49%), Pomona (24.41%) and Hardjo (23.00%) were the most prevalent in the samples examined, as described in Table 1. Other serovars such as Canicola, Grippotyphosa, Autumnalis, Bratislava and Pyrogenes were also reagents in some samples. There were no positive samples for the serovars Bataviae, Shermani, Sentot, Guaricura, Whitcombi, Andamana and Patoc (not included in Table 1).

Among the positive samples, 45.54% (97/213) were reagents to only one serovar, and 32.39% (69/213) were reagents to two serovars. In comparison, 15.49% (33/213) were reagents to three serovars, 4.69% (10/213) were reagents to four serovars, and 1.88% (4/213) were reagents to five different serovars. The distribution of leptospirosis cases and the respective serovars found on each property are illustrated in Table 2.

Risk factors such as the presence of dogs and cats on the property, co-grazing of buffaloes with species like swine, bovines, horses or sheep and reports of reproductive problems in the herds were analyzed (Table 3). It was found that buffaloes raised in properties with the presence of dogs (p=0.005) and cats (p=0.002) and co-grazing with swine (p=0.001) were more likely to be seropositive to *Leptospira* spp. Co-grazing with bovine, horses and sheep, and reproductive problems

reported in the properties didn't have statistical significance (Table 4).

Among the properties from which samples were collected, 83.33% (15/18) had at least one animal seropositive for leptospirosis, while 16.67% (3/18) had no animal reacting to the MAT test for leptospirosis. In Table 5, it was possible to observe that the larger the herd, the higher the frequency of positive buffaloes for *Leptospira* spp.

DISCUSSION

Leptospirosis in buffaloes has been reported in several countries through serological studies that demonstrated different prevalences (Konrad et al. 2013, Alamuri et al. 2019, Olmo et al. 2019, Guedes et al. 2020a). The frequency of leptospirosis in buffaloes in the state of Paraná found in the present study was 77.17% (213/276), a high result close to the 80% (164/205) found by Viana et al. (2009) in Pará and 70.58% (216/306) found by Carvalho et al. (2015) in Maranhão. The frequency found was higher than those found in other studies conducted in different states of Brazil, such as 28.5% (35/123) in Pernambuco (Oliveira et al. 2018), 27.9% (38/136) in Paraíba (Pimenta et al. 2019), 67.7% (86/127) in buffaloes in Pará (Silva et al. 2009) or 56.25% (18/32) in Minas Gerais (Mendes et al. 2018). It is also important to consider that frequency of leptospirosis can be affected by the number of serovars used in MAT testing (Adesivun et al. 2009).

The serovar with the highest frequency among the seropositive buffaloes tested in this study was Icterohaemorrhagiae, with 92.49% of seropositive animals (197/213). This result is considered high when compared to the frequency of the serovar Icterohaemorrhagiae in other studies of leptospirosis frequency in buffaloes in Brazil, where the frequency was 24.5% (53/216) in Maranhão (Carvalho et al. 2015), 33.6% (51/152) in São Paulo (Langoni et al. 1999) and 5% (2/40) in the city of Belém (Rocha et al. 2019). In other countries such as India (21.38%, 46/215) (Alamuri et al. 2019), Tanzania (5.26%, 2/38) (Assenga et al. 2015), Turkey (1.08%, 1/30) (Kenar & Ozdemir 2013) or Iran (8.1%, 3/37) (Mohammad 2014) the prevalence of serovar Icterohaemorrhagiae was also lower than that obtained in the present study.

The main hosts or reservoirs of the serovar Icterohaemorrhagiae are rodents, which suggests that they play a fundamental role in transmitting leptospirosis to the buffaloes used in this study (Campos et al. 2017). This raises the hypothesis that rodents have access mainly to food offered to buffaloes but also that

Table 1. Frequency of each sorovar tested by MAT test among the buffaloes that tested positive (213/276) for *Leptospira* spp. in this study

1			5		
Serovar	Rea	agent	Not reagent		
Serovar	Ν	%	Ν	%	
Icterohaemorrhagiae	197	92.49%	14	6.49%	
Pomona	52	24.41%	161	75.59%	
Hardjo	49	23.00%	164	77.00%	
Autumnalis	39	18.31%	174	81.69%	
Grippotyphosa	30	14.08%	183	85.92%	
Bratislava	22	10.33%	191	89.67%	
Canicola	3	1.41%	210	98.59%	
Pyrogenes	2	0.94%	211	99.06%	

they may contaminate the wetlands or the environment where these buffaloes live together, especially in places close to urban areas or with poor sanitary conditions.

Icterohaemorrhagiae serovar has also been reported with high frequency in horses (Rizzo et al. 2022). Therefore, the high frequency of buffaloes positive to this serovar could be associated with the contact or co-grazing of horses on the property since 66.67% (12/18) of the properties of the present study had horses on the property. This serovar is also one of the most predominant serovars found in dogs and pigs (Pinto et al. 2017), which suggests that they could also be the source of infection of this serovar to buffaloes since 88.89% (16/18) of the properties have dogs, and 16.67% (3/18) have co-creation with swine (Table 3).

The second most common serovar found in seropositive buffaloes from the state of Paraná was the Pomona serovar, with 24.41% (52/213) positive animals. This serovar has also been reported to infect buffaloes in Brazil, and studies show a prevalence of 29.41% (90/216) in Maranhão (Carvalho et al. 2015), 16.3% (109/665) in Amazonas (Guedes et al. 2020b), 31.6% (12/38) (Pimenta et al. 2019).

The Pomona serovar has the pig as its main host (Faine et al. 1999, Pimenta et al. 2019), which suggests that infected

bubaline may have contact with pigs, the latter being its source of transmission. Interestingly, the herds where there was co-grazing of buffaloes and swine (Herd 5, 8 and 9) didn't have such high seroprevalence rates for the Pomona serovar, while the herd (Herd 12) with the highest frequency for Pomona serovar didn't report contact with swine or any of the animals considered as possible risk factors for the herd (Table 3 and 4).

Hardjo serovar was the third most frequent serovar between seropositive buffaloes in the state of Paraná, with 23.00% (49/213) seroreagent animals for this serovar. Some studies consider this the most frequent serovar found in bubaline herds in Brazil, with a prevalence of 43.4% (165/381) and 33.6% (51/152) in São Paulo (Langoni et al. 1999, Favero et al. 2002), 23,25% (20/86) and 76,44% (2901/3795) in Pará (Silva et al. 2009, Dias et al. 2014), 43.2% (15/35) in Pernambuco (Oliveira et al. 2018), and 20.4% (18/88) in Marajó Island (Oliveira et al. 2013). Thus, the prevalence of Hardjo serovar in bubaline from the state of Paraná can be considered lower than in other states and regions of Brazil.

The high frequency of Hardjo serovar in buffaloes may be related to its similarity to cattle, considered a maintenance host for this serovar (Chiareli et al. 2012, Chideroli et al. 2017).

m 11 0 11 1 C		
Table 2 Number of 1	positive animals for certain serovar in each property	v according to municipality and mesoregion
Tuble Entumber of	positive animals for certain scrovar in cach property	, according to municipality and mesor egion

MMNH ^a	NAH ^b	ICT ^c	CAN ^d	POM ^e	GRIP ^f	HRJ ^g	AUT ^h	BRA ⁱ	PYR ^j
Oriental Center									
Ponta Grossa/herd 1	22	13	-	8	1	7	1	3	1
Carambeí/herd 2	20	11	-	2	-	-	-	2	-
South Center									
Laranjeira do Sul/herd 3	10	-	-	-	1	-	-	-	-
Laranjeira do Sul/herd 4	6	1	-	-	-	-	-	-	-
Curitiba (Metropolitan)									
Campina grande do Sul/herd 5	31	29	1	5	-	5	5	5	1
Adrianópolis/herd 6	8	8	-	-	-	8	2	-	-
Morretes/herd 7	23	20	-	4	5	3	-	-	-
Cerro Azul/herd 8	15	11	-	-	2	7	2	-	-
Cerro Azul/herd 9	11	9	-	1	2	-	4	2	-
Rio Branco do Sul/herd 10	30	27	-	1	5	15	6	2	-
Rio Branco do Sul/herd 11	21	19	-	1	2	-	3	-	-
Rio Branco do Sul/herd 12	26	25	1	21	5	2	9	7	-
Campo Largo/herd 13	7	7	1	7	4	1	3	1	-
North Center									
Uniflor/herd 14	11	9	-	1	2	-	2	-	-
North Pioneer									
Pinhalão/herd 15	7	-	-	-	-	-	-	-	-
Ibaiti/ herd 16	8	-	-	-	-	-	-	-	-
Northeast									
São Mateus do Sul/herd 17	14	8	-	1	1	1	2	-	-
Teixeira Soares/herd 18	6	-	-	-	-	-	-	-	-
TOTAL	276	197	3	52	30	49	39	22	2

(-) Did not had any reagent animal; ^a Mesoregion, municipality, number of the herd, ^b number of animals of the herd, ^c Iccterohaemorrhagiae, ^d Canicola, ^e Pomona, ^f Grippotyphosa, ^g Hardjo, ^h Autumnalis, ⁱ Bratislava, ^j Pyrogenes.

	grazing	with another sp	ecies in each farm	and reports of reproductive proble	ms	
MMNH ^a	NAH ^b	NBPALS ^c	PASIP ^d	CAS ^e	RP ^f	
			Oriental Ce	enter		
1*	22	18	Dogs	Bovine, horses and sheeps	Yes	
2*	20	14	Dogs	Bovine and horses	Yes	
			South Cer	iter		
3*	10	1	Dogs	Bovine, horses and sheeps	No	
4*	6	1	Dogs	Bovine, horses and sheeps	No	
			Curitib	a		
5*	31	29	Dogs and cats	Swine	No	
6*	8	8	-	-	No	
7*	23	22	Dogs and cats	-	No	
8*	15	14	Dogs and cats	Horses, sheeps and swine	No	
9*	11	10	Dogs and cats	Horses and swine	Yes	
10*	30	28	-	-	No	
11*	21	19	Dogs and cats	Horses	No	
12*	26	25	Dogs	-	Yes	
13*	7	7	Dogs and cats	-	No	
			North Cer	nter		
14*	11	9	Dogs and cats	Bovine and horses	Yes	
			North Pio	neer		
15*	7	0	Dogs	Bovine, horses and sheeps	Yes	
16*	8	0	Dogs	Bovine and horses	No	
			Northea	ist		
17*	14	8	Dogs and cats	Bovine, horses and sheeps	No	
18*	6	0	Dogs and cats	Bovine and horses	No	
TOTAL	276	213				

Table 3. Number of animals positive to at least one serovar, presence of another animal species in the properties or co-
grazing with another species in each farm and reports of reproductive problems

^a Mesoregion, municipality, number of the herd, ^b number of animals of the herd, ^c number of buffaloes positive to at least one sorovar, ^d Presence of another species on the property, ^e co-grazing with another specie, ^f reproductive problems reported; (-) Did not had presence of another animal species in the property or co-grazing with another species; * 1 = Ponta Grossa, 2 = Carambeí, 3 and 4 = Laranjeira do Sul, 5 = Campina Grande do Sul, 6 = Adrianópolis, 7 = Morretes, 8 and 9 = Cerro Azul, 10, 11 and 12 = Rio Branco do Sul, 13 = Campo Largo, 14 = Uniflor, 15 = Pinhalão, 16 = Ibaiti, 17 = São Mateus do Sul, 18 = Teixeira Soares.

Table 4. Frequency (%) of positive and negative buffaloes for <i>Leptospira</i> spp. and odds ratio (OR – 95%) according with					
exposure or not to the risk factors					

Presence of dogs on the property Yes No Presence of cats on the property Yes No	n 177 36 118	% 74.37% 94.74%	n 61	% 25.63%	— OR (95%)	<i>p</i> -value
No Presence of cats on the property Yes	36		61	25.63%	0.1((0.02,0,(0))	
Presence of cats on the property Yes		94.74%		,,	0.16 (0.03-0.69)	0.005
	110		2	5.26%		
No	110	84.89%	21	15.11%	2.48 (1.38-4.48)	0.002
	95	69.34%	42	30.66%		
Co-grazing with swine Yes	53	92.98%	4	7.02%	4.89 (1.69-14.09)	0.001
No	160	73.06%	59	26.94%		
Co-grazing with bovines Yes	51	49.04%	53	50.96%	0.06 (0.03-0.12)	4.724
No	162	94.18%	10	5.82%		
Co-grazing with horses Yes	94	62.25%	57	37.75%	0.08 (0.03-0.20)	7.955
No	119	95.20%	6	4.80%		
Co-grazing with sheeps Yes	42	56.76%	32	43.24%	0.24 (0.13-0.43)	1.001
No	171	84.65%	31	14.35		
Reproductive problems Yes	76	78.35%	21	21.64%	1.11 (0.61-2.01)	0.732
No	137	76.54%	42	23.46%		

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	7	7

	Number of buffaloes in the herd	Number of herds	erds Number total in the herds ——		Positive		Negative		
		Number of nerus	Number total in the nerus	n	%	n	%		
	6-10	7	52	17	32.69%	35	67.31%		
	11-20	5	71	55	77.46%	16	22.54%		
	21-30	5	122	112	91.80%	10	8.20%		
	31-40	1	31	29	93.55%	2	6.45%		
	TOTAL	18	276	213	-	63	-		

Table 5. Frequency of positive and negative buffaloes for Leptospira spp. according to the number of buffaloes in the herds

In these animals, the disease develops in the chronic and subclinical form, in which the animals persist, transmitting the disease through urine and reproductive tract secretions (Lilenbaum & Martins 2014). The infection of buffaloes may be associated with their proximity and high interaction with cattle, making them also reservoirs of this serovar (Assenga et al. 2015, Oliveira et al. 2018, Guedes et al. 2020b).

The possible risk factors associated with the occurrence of leptospirosis in buffaloes with significant differences in this study were the presence of dogs (p=0.005) and cats (p=0.002) and co-grazing with swine (p=0.001). The presence of another species in the property in contact with the herd is one of the risk factors for leptospirosis (Paixão et al. 2016, Chadsuthi et al. 2018). Dogs are frequently used for herd management, and the presence of dogs can be considered a relevant risk factor once they can disseminate the agent in the property (Paixão et al. 2016). Cats may be an incidental host of many serovars of leptospirosis, being able to be a carrier and transmit the disease to other species (Azócar-Aedo et al. 2014). Another main risk factor associated with seropositivity for leptospirosis is co-grazing with another species, mainly with pigs, as has also been demonstrated by Lilenbaum & Souza (2003).

Although there were reports of reproductive problems, this was not considered a significant risk factor in the present study. Genital leptospirosis normally occurs silently and is often diagnosed in herds with reproductive problems such as repeated estrus, low fertility or abortions (Galiero 2007, Marianelli et al. 2007, Ijaz et al. 2020, Loureiro & Lilenbaum 2020). The fetuses, placenta and fluids of carrier animals, even if apparently healthy, can also be a source of infection to other animals and humans (Balamurugan et al. 2016).

When comparing the properties, 83.33% (15/18) of the properties where samples were collected from had at least one seropositive animal for *Leptospira* spp. It is noteworthy that the two properties in the North Pioneer mesoregion had no cases of positive animals for leptospirosis, which may suggest that the prevalence in this region is lower than in other regions. It's also relevant that the larger the herd, the higher the frequency of seropositive buffaloes for leptospirosis in this study, which may suggest that small properties have a lower rate of spread of the agent, and this could be due to lower animal density, management, lower infection pressure or other external factors.

This is the first study in the literature evaluating the prevalence of leptospirosis in buffaloes in Paraná. For this reason, scant data are available about the epidemiological situation of buffaloes in this state concerning the disease until now. In cattle, a study by Hashimoto et al. (2012) determined that the prevalence of leptospirosis in beef and dairy cattle

from Paraná was 34.41% (647/1800), a rate lower than that found for buffaloes in this study.

The high prevalence found in this study shows that leptospirosis is present in infecting buffaloes in the state of Paraná and may be related to management, environmental conditions, herd health status, lack of vaccination and routine diagnosis or control, and failure to treat or dispose of seropositive animals (Higino et al. 2012). This demands attention regarding public health since these animals are used as a source of meat or milk and have contact with humans (Guedes et al. 2020a).

For this reason, it would be important to include leptospirosis in surveillance programs to develop strategies to control the transmission of the disease in buffaloes herds and other animals, as identify the serovars circulating in a given region and promote the vaccination of herds for the serovars present in this respective region or herd (Ellis 2015, Alamuri et al. 2019, Loureiro & Lilenbaum 2020). Isolation and treatment of seropositive animals are also indicated (Levett 2001). It's also recommended to control the rodent population, especially in places susceptible to flooding or inundation, to pay attention to the animal's water source, as well as to avoid the joint breeding of different species and high animal density in the same area (Nardi Júnior et al. 2010, Saito et al. 2013, Paixão et al. 2016, Chadsuthi et al. 2018). These topics are important once humans, especially veterinarians and farmers, who care for or manage these buffaloes and other farm animals, can be infected since it's a zoonosis (Genovez 2009).

CONCLUSIONS

This study seems to be the first to determine the frequency of leptospirosis in buffaloes from the state of Paraná and their respective serovars prevalence.

The frequency of buffaloes with leptospirosis in Paraná is considered high. The main serovars circulating in this population are Icterohaemorrhagiae, Hardjo and Pomona, which suggests contact of these buffaloes with rodents, pigs and other wild animals.

Control and prevention measures are necessary to prevent the transmission of leptospirosis in buffalo herds, as well as to humans and other animal species in the state of Paraná since it is a public health issue.

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