[Acta Veterinaria Brasilica September 13 (2019) 126-134]

Acta Veterinaria Brasilica

Journal homepage: https://periodicos.ufersa.edu.br/index.php/acta/index

Original article

Epidemiological aspects of leptospirosis in cattle herds from indigenous reserves

Aspectos epidemiológicos da leptospirose em rebanhos bovinos de reservas indígenas

Adalgoberto Santana Guedelha[1]*, Daniela da Silva Alves Guedelha[1], Anna Karoline Amaral Sousa[1], Helder de Moraes Pereira[2], Danilo Cutrim Bezerra[3], Hamilton Pereira Santos[4], Nancyleni P. Chaves Bezerra[5]

[1] Professional Postgraduate Program in Animal Sanitary Defense (Masters Course), Estadual University of Maranhão, São Luis, Maranhão, Brazil.
[2] Department of Zootechnics, Estadual University of Maranhão, São Luis, Maranhão, Brazil.
[3] Department of Zootechnics, Estadual University of Maranhão, São Luis, Maranhão, Brazil.
[4] Professional Postgraduate Program in Animal Sanitary Defense (Masters Course), Department of Pathology, Estadual University of Maranhão, São Luis, Maranhão, Brazil.
[5] Professional Postgraduate Program in Animal Sanitary Defense (Masters Course), Department of Fisheries Engineering, Estadual University of Maranhão, São Luis, Maranhão, Brazil

A R T I C L E I N F O

Article history
Received 17 January 2019
Accepted 16 June 2019

Keywords:
Leptospira
Indigenous lands
Cattle
Risk factors

A B S T R A C T

The aim of the current study is to evaluate the epidemiological aspects of leptospirosis in cattle herds from indigenous reserves. Serum samples from cows - not vaccinated against the disease and distributed in five indigenous reserves of Barra do Corda region, Maranhão State - were subjected to the microscopic agglutination technique. An epidemiological questionnaire focused on investigating the risk factors associated with *Leptospira* spp. infection was applied in each indigenous reserve. Of the 300 analyzed samples, 80% presented positive reactions with titers equal to or higher than 1:100. Reactive animals were identified in all (100%) indigenous reserves. The most often found serovars of the *Leptospira* spp. complex are herein presented in descending order: Shermani, Guaricura, Sentot, Djasiman, Patoc, Hardjo C.T.G, Hardjo Bovis, Hardjo, Hardjo Prajitno, Wolffi, Hebdomadis, Adamanda and Icterohemorragiae. Among the herein analyzed epidemiological variables, the inadequate disposal of miscarried fetuses and placental remnants, the incidence of miscarriages in the last 12 months, the calf birth concentration in the rainy season and the lack of corrals presented statistically significant association ($P < 0.05$) with the risk of infection. Results indicated high prevalence of *Leptospira* spp. infection in cattle bred in the indigenous reserves of Barra do Corda. Variables such as reproductive clinical signs, environmental factors, aspects of local infrastructure and sanitary management should be taken into consideration to help preventing the incidence of leptospirosis in indigenous reserves.

Palavras-chave:
Leptospira
Reservas indígenas
Gado
Fatores de risco

R E S U M O

O estudo teve como objetivo avaliar os aspectos epidemiológicos da leptospirose em rebanhos bovinos de reservas indígenas. Amostras de soro de fêmeas bovinas não vacinadas contra a doença e provenientes de cinco reservas indígenas da regional de Barra do Corda, Estado do Maranhão foram submetidas à técnica de soroaglutinação microscópica. Em cada reserva aplicou-se um questionário epidemiológico para investigar os fatores de risco que poderiam estar associados à infecção pela *Leptospira* spp. Das 300 amostras analisadas, 80% apresentaram reações positivas, com títulos iguais ou superiores a 1:100. Foram identificados animais reagentes em 100% das reservas indígenas. Os sorovares do complexo *Leptospira* spp. mais frequentes em ordem decrescente foram: Shermani, Guaricura, Sentot, Djasiman, Patoc, Hardjo C.T.G, Hardjo Bovis, Hardjo, Hardjo Prajitno, Wolffi, Hebdomadis, Adamanda e Icterohemorragiae. Das variáveis epidemiológicas, a não destinação correta aos fetos abortados e restos placentários, ocorrência de abortamento nos últimos 12 meses, concentração de

* Corresponding author: aged.barradocorda@hotmail.com

http://dx.doi.org/10.21708/avb.2019.13.3.8373

nascimento de bezerros no período chuvoso e ausência de curral apresentaram associação estatística significativa (P<0,05) ao risco de ocorrência da infecção. Os resultados do estudo indicam que a infecção por *Leptospira* spp. encontra-se presente em bovinos das reservas indígenas de Barra do Corda em alto percentual. As variáveis relacionadas à sinais clínicos reprodutivos, à fatores ambientais, aspectos de infraestrutura local e de manejo sanitário devem ser considerados na prevenção da leptospirose em reservas indígenas.

INTRODUCTION

Cattle breeding systems are focused on producing and reproducing cattle in order to meet human dietary needs, as well as to enable economic gains. According to Pereira (1990), the exploitation of cattle farming in indigenous lands is based on political and economic goals. The political goals lie on assuring the ownership of the land and on creating conditions for an expansion and occupation regime similar to the one implemented by settlers: land with cattle means land with owner. The economic goals are to assure richer diets based on products such as meat and milk, as well as to enable economic development through the production and commercialization of by-products such as cheese and leather.

Mariano (2011) reported that indigenous cattle herds present low animal performance due to lack of or deficient food supplementation, and zootechnical and sanitary control. With respect to the health of herds bred in indigenous lands, sick animals are identified and treated through the help of technicians from institutions such as the National Indian Foundation, the Ministry of Agriculture, Livestock and Supply and state animal health-defense agencies, which are summoned when animals present some disorder. The most applied vaccines are the ones against foot-and-mouth disease and brucellosis; other diseases such as rabies, tuberculosis, botulism and leptospirosis are not often taken into consideration.

Leptospirosis is a neglected infectious disease of global importance caused by pathogenic spirochetes of the genus Leptospira (NAGEL et al., 2019; TEIXEISRA et al., 2019). The disease is endemic and represents serious risks to Public Health in Brazil. The country presents physiographic aspects favorable to the dissemination and endemicity of leptospirosis, among them: its typical temperature and humidity conditions, and the presence of wild fauna, which constitute potential reservoirs (BRASIL, 1995; JORGE et al., 2017).

Leptospirosis is characterized by a broad spectrum of susceptible species. Human beings, domestic animals and wildlife species are susceptible to a wide variety of serovars (VIEIRA; LINLENBAUM; 2017). The disease is transmitted directly following contact with infected urine and other body fluids or indirectly after contact with water or soil contaminated with infected urine (GARBA et al., 2018).

Among all domestic animals, cattle are the main responsible for the maintenance and introduction of leptospirosis in several properties; thus, they are seen as important leptospirosis disseminators in humans. Bovine animals can be infected by any pathogenic leptospire serotypes found in the environment (ARAÚJO et al., 2005).

The spread of leptospirosis in cattle herds is characterized by the presence of sick animals or asymptomatic carriers who eliminate the bacterium through urine, cervicovaginal discharges, miscarried fetuses and placenta, and it keeps the disease endemic in the herd (HASHIMOTO et al., 2012; COELHO et al., 2014). Other factors such as the existence of *Leptospira* spp. serovars in the region, the simultaneous breeding of animals belonging to distinct species, the presence of wild animals, environmental and climatic conditions, besides handling strategies, can influence the contact between the cattle and the microorganism (OLIVEIRA et al., 2009).

Therefore, the current research is relevant to indigenous and non-indigenous communities, since it gathered primary data not yet investigated or analyzed, besides contributing to the social and political debate focused on indigenous communities. Thus, given the insufficient information about the health of herds bred in indigenous lands in Maranhão State, the aim of the current study was to evaluate the epidemiological aspects of leptospirosis in cattle herds from indigenous reserves.

MATERIALS AND METHODS

Study Site

The study site comprised five indigenous reserves located in Barra do Corda, Arame, Itaipava do Grajaú, Grajaú and Jenipapo dos Vieiras counties, which belong to Barra do Corda region. This region is located in the central area of Maranhão State, approximately 436 km away from the capital city São Luís - MA. Maranhão State holds a cattle herd of 7,488,943 animals; 4,918 of this total are bred by indigenous peoples in their reserves and 43.28% (n = 2,129) of them are bred in Barra do Corda region (MARANHÃO, 2016).

Sample Delimitation

Firstly, it is necessary clarifying that the concept of "rural property" in the agricultural defense field goes beyond the idea of fenced lands - such as farms, small farms or ranches - where some agricultural activity is practiced.

Property is an epidemiological unit that may consist of one or more breeding units (or production units), which are subject to the same health risks. Thus, other rural complexes such as villages, indigenous reserves, settlements or even countryside headquarters, whose households do not have breeding areas and animals often graze in communal areas (flooded fields, coconut groves or mangrove forests), also fit this new concept.

Sampling procedures were carried out in two stages. Firstly, a pre-established number of herds representing the primary sampling units was randomly selected. A pre-established number of animals (secondary units) within the primary units were randomly sampled. The number of herds was calculated based on the confidence level of the result, on the desired precision level and on the expected prevalence value, through the formula for simple random sampling, according to Noordhuizen et al. (1997).

The random selection of herds in each indigenous reserve was based on the records of these reserves in the database of Barra do Corda Regional Unit of Maranhão State Agency for Agricultural and Livestock Defense (AGED-MA - Agência Estadual de Defesa Agropecuária do Maranhão). The reserves and herds in each county were numbered and their data were stored in Microsoft Excel 2000® spreadsheets.

The sample planning of the secondary units was focused on estimating the minimum number of animals to be examined in each indigenous reserve/property. The number of animals selected in each herd was set in the Herdacc® software version 3 (University of Guelph), by assuming at least 50% disease prevalence (CASTRO et al., 2008) and 95% probability of detecting at least one reactive animal.

A similar number of animals was selected in each indigenous reserve based on the simple random sampling technique; 60 animals were sampled in each one of them.

Three hundred (300) serum samples collected from female beef cattle (age > 24 months) selected in five indigenous reserves, not vaccinated against leptospirosis, were herein analyzed. Male animals and cows in the puerperium (fifteen days before or after calving) were not included in the current study. The distribution of counties, reserves and the total number of samples are listed in Table 1.

Table 1 – Number of samples distributed per county, indigenous reserve and herd in Barra do Corda regional unit – Maranhão Sate, Brazil.

Regional Unit	County	N. of Indigenous Reserves	N. of Herds	N. of Samples
	Arame	01	01	60
	Barra do Corda	01	01	60
Barra do Corda	Grajau	01	01	60
	Itaipava do Grajaú	01	01	60
	Jenipapo dos Vieiras	01	01	60
TOTAL		05	05	300

Blood Sample Collection

Blood samples were collected from November 2016 to May 2017, when the owners of the animals received information about the study, leptospirosis and about the risks it poses to animal and human health, as well as about economic losses resulting from the disease. Indigenous breeders participating in the study signed the Free and Informed Consent Form.

The blood was collected by means of jugular vein puncture using sterile disposable needle and previously identified vacuum tube. The collected material was placed in a styrofoam box filled with reusable ice and transported to the headquarters of Barra do Corda Regional Unit, where it was centrifuged. The resulting serum was stored in plastic microtubes and frozen at -20°C. Next, the microtubes were sent to the Laboratory for the Diagnosis of Infectious Diseases at Maranhão State University (UEMA - Universidade Estadual do Maranhão), for laboratory diagnosis purposes.

The current study was approved by the Ethics Committee on Animal Experimentation (EAEC – Comissão de Ética na Experimentação Animal) of UEMA Veterinary School - protocol N. 27/2015.

Laboratory Diagnosis

Blood sera were tested for anti-*Leptospira* agglutinins against 31 serovars of the *Leptospira* spp. complex coming from the bank of Federal University of Pará/UFPA and kept by the UEMA Laboratory for the Diagnosis of Infectious Diseases through the Microscopic Agglutination Technique (MAT), according to standards set by the Ministry of Health (OIE, 2010). Each serum sample was initially diluted in buffered saline solution at ratio 1:100, pH 7.2, and tested against the serovars listed in Table 2. Samples presenting agglutination equal to or higher than 50 were considered reactive.

Samples reacting to the initial titer (sorting) were tested again in order to define the antibody titer for each serovar by using increasing dilutions from 1:100 to 1:800. Samples presenting titer equal to or higher than 100, as well as 50% agglutination or disappearance of field cells under darkfield microscopy, were considered reactive (BRASIL, 1995). Reactive sera were titrated in

geometric series of four two-fold dilutions; the titer was recorded as the reciprocal of the highest serum dilution showing agglutination.

Table 2 – Collection of antigens of the Leptospira spp. complex used in the Microscopic Agglutination Test (MAT).

Serogroup	Serovar
Australis	Australis; Bratislava
Autumalis	Autumnalis
Ballum	Butembo; Castellonis
Batavia	Bataviae
Canicola	Canicola
Calledoni	Whitcombi
Cynopteri	Cynopteri
Gripotyphosa	Gripotyphosa
Hebdomadis	Hebdomadis
Icteriohaemorragie	Copenhageni; Icteriohaemorragiae
Javanica	Javanica
Panama	Panama
Pomona	Pomona
Pyrogenes	Pyrogenes
Serjroe	Hardjo; Wolfi, Guaricura, Hardjo Bovis, Hardjo Prajitno, Hardjo C.T.G., Hardjo Miniswajizak
Shermani	Shermani
Terassovi	Terassovi
Andamana	Andamana
Semaranga	Patoc
Djasiman	Sentot, Djasiman

Risk Factors

An epidemiological questionnaire was applied in each indigenous reserve to collect information about animal handling and sanitary status. The questionnaire comprised questions about local infrastructure, as well as about dietary, sanitation and reproductive management.

Univariate analysis was performed through Fisher's exact test in order to investigate the association between each possible risk factor and the dependent variable (*Leptospir*a-free or -infected herd). The significance level was set at 5% and the confidence intervals, at 95%.

RESULTS

Three hundred (300) serum samples from cows bred in indigenous reserves in Maranhão State were subjected to the MAT technique in order to investigate the prevalence of *Leptospira* spp. Eighty percent of them (80% - n = 240) presented positive reactions with titers equal to or higher than 1:100, whereas 20% (n = 60) of them tested negative for all herein adopted serovars.

Reactive animals were identified in 100% (n = 5) of the herein evaluated counties and indigenous reserves. Table 3 summarizes the serological results per county, indigenous reserve and animal.

Table 3 – Serological results of anti-*Leptospira* spp. antibodies per county, herd and animal in indigenous reserves of Barra do Corda regional unit - Maranhão State, Brazil.

Regional Unit	County	Herds/Indigenous Reserve			Cattle		
		N	Reactive	%	N	Reactive	%
Barra do Corda	Arame	01	01	100	60	54	90
	Barra do Corda	01	01	100	60	60	100
	Grajau	01	01	100	60	58	96.66
	Itaipava do Grajau	01	01	100	60	21	35
	Jenipapo dos Vieiras	01	01	100	60	47	78.33
Total		05	05	100	300	240	80%

The serovars of the *Leptospira* spp. complex most often found in the evaluated samples were Shermani (110/300; 36.67%), Guaricura (105/300; 35%), Sentot (94/300; 31.33%), Djasiman (92/300; 30.67%), Patoc (92/300; 30.67%), Hardjo C.T.G (89/300; 29.67%), Hardjo Bovis (83/300; 27.67), Hardjo (80/300; 26.67%), Hardjo Prajitno (79/300; 26.34%), Wolffi (68/300; 22.61%), Hebdomadis (53/300; 17.67%), Adamanda (43/300; 14.33%) and Icterohemorrhagiae (39/300; 13%), as shown in Table 4. The evaluated countries presented different prevailing serovars (Table 5).

Serum samples from Arame County presented 90% reaction to least one of the 31 serovars used in the current study; Djasiman (31/60; 51.67%) and Guaricura (30/60; 50%) were the most frequent ones. Barra do Corda was the only county in the current study where samples recorded 100% positivity for at least one of the tested serovars; Hardjo C.T.G (42/60; 70%) recorded the highest prevalence of it. With respect to Grajaú County, 96.66% of the samples reacted to at least one out of 31 serovars; Shermani (41/60; 68.00%) was the most frequent one. Animals from Itaipava do Grajaú County recorded the lowest positivity rate (21/60; 35%); Canicola (7/60, 11.67%) was the serovar recording the highest prevalence. The indigenous reserve of Jenipapo dos Vieiras recorded reaction rate 78.33% (47/60); Butembo (23/60; 38.33%) and Hardjo (22/60; 36.67%) were the most frequent serovars.

The epidemiological questionnaire allowed investigating the risk factors. The univariate analysis showed that variables such as inadequate disposal of miscarried fetuses and placental remnants, incidence of miscarriages in the last 12 months, calf birth concentration in the rainy season and lack of corrals presented statistically significant association ($P<0.05$) with the risk of *Leptospira* spp. infection in the evaluated area (Table 6).

Table 4 – *Leptospira* spp. serovars identified in the blood serum of cows bred in indigenous reserves of Barra do Corda regional unit – Maranhão State, Brazil.

Serovar	Reactive	Percentage (%)
Shermani	110	36.67%
Guaricura	105	35.00%
Sentot	94	31.33%
Djasiman	92	30.67%
Patoc	92	30.67%
Hadjo CTG	89	29.67%
Hadjo Bovis	83	27.67%
Hadjo	80	26.67%
Hadjo Prajitno	79	26.33%
Wolffi	68	22.67%
Hebdomadis	53	17.67%
Adamanda	43	14.33%
Icterohemorragiae	39	13.00%
Whitcombi	37	12.33%
Butembo	31	10.33%
Canícola	25	8.33%
Autuminallis	24	8.00%
Taransovi	20	6.67%
Grippotyphosa	18	6.00%
Australlis	17	5.67%
Hardjo Miniswajizak	16	5.33%
Copenhageni	16	5.33%
Nupezo	10	3.33%
Cynopte	09	3.00%
Javanica	08	2.67%
Pyrogenes	08	2.67%
Panamá	07	2.33%
Pomona	06	2.00%
Castelonis	05	1.67%
Batavae	02	0.67%
Brastilava	01	0.33%

Table 5 – Distribution of antibodies of *Leptospira* spp. serovars found in herds bred in the indigenous reserves of Barra do Corda regional unit, Maranhão State, Brazil.

Serovar	Counties									
	Arame		Barra do Corda		Grajau		Itaipava do Grajau		Jenipapo dos Vieiras	
	React.	%	React.	%	React.	%	React.	%	React.	%
Shermani	22/60	36.67%	39/60	65.0%	41/60	68.0%	01/60	01.67%	07/60	11.6%
Guaricura	30/60	50.00%	31/60	51.6%	37/60	61.6%	00/60	00.00%	07/60	11.6%
Sentot	27/60	45.00%	22/60	36.6%	31/60	51.6%	01/60	01.67%	13/60	21.6%
Djasiman	31/60	51.67%	27/60	45.0%	30/60	50.0%	00/60	00.00%	04/60	06.6%
Patoc	28/60	46.67%	37/60	61.6%	27/60	45.0%	00/60	00.00%	00/60	00.0%
Hadjo CTG	20/60	33.33%	42/60	70.0%	25/60	41.6%	00/60	00.00%	02/60	03.3%
Hadjo	22/60	36.67%	19/60	31.6%	11/60	18.3%	06/60	10.00%	22/60	36.6%
Hadjo Bovis	19/60	31.67%	29/60	48.3%	30/60	50.0%	00/60	00.00%	05/60	08.3%
Hadjo Prajitno	15/60	25.00%	38/60	63.3%	24/60	40.0%	00/60	00.00%	02/60	03.3%
Wolffi	17/60	28.33%	25/60	41.6%	14/60	23.3%	03/60	05.00%	09/60	15.0%
Hebdomadis	18/60	30.00%	24/60	40.0%	07/60	11.6%	00/60	00.00%	04/60	06.6%
Adamanda	12/60	20.00%	12/60	20.0%	15/60	25.0%	01/60	01.67%	03/60	05.00%
Icterohemorragiae	13/60	21.67%	15/60	25.0%	07/60	11.6%	01/60	01.67%	03/60	05.00%
Whitcombi	09/60	15.00%	10/60	16.6%	17/60	28.3%	00/60	00.00%	01/60	01.67%
Butembo	03/60	05.00%	01/60	01.6%	03/60	05.0%	01/60	01.67%	23/60	38.33%
Canícola	01/60	01.67%	02/60	03.3%	04/60	06.6%	07/60	11.67%	11/60	18.33%
Autuminallis	03/60	05.00%	03/60	05.0%	04/60	06.6%	03/60	05.00%	11/60	18.33%
Taransovi	02/60	03.33%	05/60	08.3%	06/60	10.0%	02/60	03.33%	05/60	08.33%
Grippotyphosa	07/60	11.67%	04/60	06.6%	05/60	08.3%	00/60	00.00%	02/60	03.33%
Australlis	02/60	03.33%	09/60	15.0%	05/60	08.3%	00/60	00.00%	01/60	01.67%
Hadjo Miniswajizak	05/60	08.33%	03/60	05.0%	08/60	13.3%	00/60	00.00%	00/60	00.00%
Copenhageni	05/60	08.33%	03/60	05.0%	05/60	08.3%	00/60	00.00%	03/60	05.00%
Nupezo	03/60	05.00%	04/60	06.6%	03/60	05.0%	00/60	00.00%	00/60	00.00%
Cynopte	01/60	01.67%	05/60	08.3%	03/60	05.00%	00/60	00.00%	00/60	00.00%

Table 5 – Distribution of antibodies of *Leptospira* spp. serovars found in herds bred in the indigenous reserves of Barra do Corda regional unit, Maranhão State, Brazil (continuação).

Javanica	03/60	05.00%	01/60	01.6%	04/60	06.6%	00/60	00.00%	00/60	00.00%
Pyrogenes	01/60	01.67%	03/60	05.0%	03/60	05.0%	01/60	01.67%	00/60	00.00%
Panamá	01/60	01.67%	0/60	00.0%	00/60	00.0%	02/60	03.33%	04/60	04.00%
Pomona	00/60	00.00%	02/60	03.3%	01/60	01.6%	00/60	00.00%	03/60	05.00%
Castelonis	01/60	01.67%	0/60	00.0%	01/60	01.6%	00/60	00.00%	03/60	05.00%
Batavae	00/60	00.00%	0/60	00.0%	00/60	00.00%	02/60	03.33%	00/60	00.00%
Brastilava	00/60	00.00%	01/60	01.6%	00/60	00.0%	00/60	00.00%	00/60	00.00%

Table 6 – Risk factors associated with *Leptospira* spp.-reactive animals kept in herds of five indigenous reserves in Barra do Corda regional unit, Maranhão State, Brazil.

Leptospirosis								
Variables		Reactive	%	Non-reactive	%	OR	CI%	P value
Disposal of miscarried fetuses and placental remnants	Buries it	114	38	06	02	0.122	0.058;0.296	<0.001*
	Does not do anything	126	42	54	18			
Miscarriages in the last 12 months	Yes	219	73	21	07	19.36	9.672;38.782	<0.001*
	No	21	07	39	13			
Season recording the highest calf birth rate	Dry	75	25	45	15	0.151	0.079;0.288	<0.001*
	Rainy	165	55	15	5			
Corrals	Yes	114	38	06	02	0.122	0.050;0.296	<0.001*
	No	126	42	54	18			
Handles animals on a daily basis	Yes	128	43	52	17	0.175	0.080;0.386	<0.001*
	No	112	37	08	03			
Source of water supplied to animals	River	139	46	41	14	0.637	0.349;1.164	0.184
	Dam	101	34	19	06			
Know any disease affecting the reproductive process	Yes	47	16	13	4	0.880	0.440;1.759	0.720
	No	193	64	47	16			

Wherein: OR= Odds ratio; CI= Confidence interval; *= statistically significant at 5% probability level.

DISCUSSION

The animals sampled in the current study came from indigenous reserves located in Barra do Corda, Arame, Grajaú, Itaipava do Grajaú and Jenipapo dos Vieiras counties. The five reserves included in the study presented the following productive characteristics: ultra-extensive breeding system, full-cycle exploration, half-breed Nellore cattle, animals fed on pasture and supplemented with common salt only, non-use of artificial insemination as reproductive biotechnology, sanitary control based on herd vaccination against foot-and-mouth disease only, using vaccines donated by the state government through Maranhão State Agency for Agricultural and Livestock Defense (AGED-MA - Agência Estadual de Defesa Agropecuária do Maranhão).

The introduction of new animals in indigenous reserves does not take into consideration the sanitary aspects of infectious diseases, besides being mainly characterized by the introduction of stallions from the region itself. Consequently, the circulation of infectious agents such as *Leptospira* spp. in the herds is highly likely.

The main serovars identified in the current study were Shermani (36.67%), Guaricura (35%), Sentot (31.33%), Djasiman (30.67%) and Patoc (30.67%). Silva et al. (2012) pointed out that some serovars, such as Shermani and Patoc, may affect public health when the environment is heavily contaminated with urine from infected animals and the infecting dose in the exposed population is high. In addition, a pre-existing immunodeficiency condition in an individual or group of individuals exposed to environments contaminated with certain serovars may generate a serious clinical condition in these individuals, even at low infecting dose.

The field trips to the reserves allowed observing the indigenous hunting habit and the maintenance of wild animals in captivity, among them: red brocket (*Mazama americana*), yellow armadillo (*Euphractus sexcinctus*), lowland paca (*Cuniculus paca*) and agouti (*Dasyprocta* spp.). This information, along with the laboratory results of the current study, reinforce the hypothesis that wild and free-living animals may be *Leptospira* spp. reservoirs for the evaluated cattle. Therefore, the identification of Shermani, Guaricura, Sentot, Djasiman, Patoc, Hebdomadis and Adamanda as the most frequent serovars indicates indirect leptospirosis transmission mechanisms, i.e., the contact between the cattle and environments contaminated with *Leptospira* spp. probably deriving from wild and free-living animals.

Several studies have demonstrated the substantial number of serovars affecting free-living animals (SILVA et al., 2010; SILVA et al., 2012; COELHO et al., 2014; CARVALHO et al., 2015; PAIXÃO et al., 2016). Opossums, deer, capybaras and other wild species may work as *Leptospira* spp. reservoirs for herds when they find satisfactory habitats (CASTRO et al., 2008; SILVA et al.,

2010). Castro et al. (2008) emphasized the importance of having a broad antigen battery composition in the microscopic agglutination technique (MAT), since serovars such as Shermani, Hebdomadis and Autumnalis, which are not often found in routine tests, may nonetheless infect herds.

Nowadays, the official recommendation at the time to apply the MAT test to investigate leptospirosis is that the adopted antigen collection must contain at least one representative per serogroup. However, the importance of including autochthonous serovars isolated in the country or region has been taken into consideration, since it could help increasing the discriminatory capacity of the test (SARMENTO et al., 2012).

Another aspect to be taken into consideration, which was already mentioned by Cubas; Silva, Catão-Dias (2014), refers to uncontrolled changes in the ecological system resulting from anthropic actions, since they rule changes in the behavior of animals searching for other ecosystems. It favors leptospires spread to new hosts and/or reservoirs, including humans. According to Genovéz (2009), the peculiarities of rural habitats, as well as the presence of wild animals, favor the transmission of leptospirosis to production animals such as cattle.

Studies have shown that Hardjo serovar is the most frequent cause of leptospiral infections affecting cattle herds worldwide (NAGEL et al., 2019) and in Brazil (COELHO et al., 2014; PAIXÃO et al., 2016). Silva et al. (2012) analyzed 2,582 serum samples from cows at reproductive age in Maranhão State and recorded 24.32% seroprevalence; the Hardjo was the most prevalent serovar (24.32%), and it was followed by Wolffi (22.00%), Patoc (12.42%), Shermani (8.85%), Grippotyphosa (8.21%) and Hebdomadis (7.35%).

Hardjo C.T.G (29.67%), Hadjo Bovis (27.67%), Hardjo (26.67%) and Hadjo Pratijno (26.34%) were quite prevalent among the herein studied serovars, fact that shows similarity between the current results and the ones recorded by the aforementioned researchers. The high rate of positive reactions to these serovars in the current study reinforces the theory by Pellegrini et al. (1999) who reported that cattle are the preferred hosts for serovar Hardjo. They also reported that the expansion of this serovar may be related to environmental factors linked to herd management, such as the inadequate disposal of placental remnants, bacterial multiplication in flooded areas and water consumption by animals in contaminated and polluted areas, which are similar situations to the ones found in the herein investigated indigenous reserves.

The Hardjo serovar recorded serum titers up to 1:800, but only in 1.25% (n = 01) of the evaluated samples. This result corroborates the study by Kirkbride (1990), who reported that titers recorded for Hardjo serovar are overall low; they are rarely higher than 1:800. According to Kirkbride (1990), when Hardjo is the most prevalent serovar, even low titers may be associated with reproductive issues and indicate infection. According to Coelho et al. (2014), the incidence of agglutinations for dilution 1:200, in most cases reinforces the infection condition among the animals. The highest agglutination rate was recorded for this dilution (1:200) in the aforementioned study.

However, the current study should not rule out the possibility of finding cross-reactivity in representatives of the same Serjroe serogroup (Hardjo, Wolfi, Guaricura, Hardjo Bovis, Hardjo Prajitno, Hardjo C.T.G., Hardjo Miniswajizak), as reported by Sarmento et al. (2012). However, definitive confirmation depends on further investigations based on sample isolation and typification.

The Wolffi serovar recorded high rate (22.67%) in the herein analyzed samples. There is agreement between results of several studies about the prevalence of serovars Hardjo and Wolffi, although they recorded different prevalence values. It reinforces the importance of both serovars, since they affect cattle and cause economic losses resulting from reproductive issues (CASTRO et al., 2008; OLIVEIRA et al., 2009).

The Icterohaemorrhagiae serovar is strongly relevant to public health, besides being often isolated in rodents (BRASIL, 2005); it recorded high prevalence (13%) in the herein evaluated cows. Different result was recorded by Silva et al. (2012), who reported low incidence of this serovar (0.48%) among reactive animals in Maranhão State.

Many wild animals, including rodents, are adapted to leptospires and do not show clinical signs or lesions. Rodents are the main leptospirosis reservoirs, since they are healthy carriers who house spirochetes in their kidneys; the living spirochetes are eliminated through the urine into the environment, thus contaminating water, soil and food (BRASIL, 1995).

The inadequate disposal of miscarried fetuses and placental remnants presented statistically significant association with *Leptospira* spp. among the variables analyzed in the current study ($P < 0.05$). Pellegrini et al. (1999) reported that the expansion of leptospirosis is associated with environmental factors concerning herd management such as the inadequate disposal of placental remnants, bacterial multiplication in flooded areas, and water consumption by animals in contaminated and polluted areas.

The incidence of miscarriages in the last 12 months also showed statistically significant association with the disease ($P < 0.05$) and behaved as risk factor (OR = 19.36) in the studied area. Serovars Hardjo and Wolffii were linked to the reproductive sphere and recorded high rate in the present study. According to Vasconcelos et al. (1997), the infection caused by serovar Hardjo has direct effect on fertilization, since it affects the function of the corpus luteum and decreases progesterone levels, whereas serovar Wolffii is often associated with Hardjo.

The calf birth concentration in the rainy season and the lack of corrals presented statistically significant association (P < 0.05) with the risk of *Leptospira* spp. infection. Speelman and Hartskeerl (2008) and Philip (2011) pointed out that animals are more likely to be infected in the rainy season because the edaphoclimatic conditions favor the dissemination of the etiological agent. As for the lack of corrals, it is possible inferring that inadequate infrastructures make it difficult to handle the animals in a proper way.

It is worth highlighting that all the herein sampled animals were previously examined at blood sample collection time and did not show any clinical signs of leptospirosis. This information, in association with the number and variety of identified serovars, emphasize the importance of asymptomatic animals for the epidemiology of leptospirosis. Campos Jr. (2006) pointed out that animals are carriers and can eliminate the causative agent indefinitely, even if they do not present clinical signs of the disease.

Leptospirosis is an occupational disease associated with certain professional groups. The health care given to the Brazilian indigenous population is an important matter that has been debated since the 1980s. Therefore, it is expected that the present study may help developing leptospirosis control strategies to be applied to indigenous reserves in Maranhão State. Studies on the local fauna should be conducted to help identifying *Leptospira* spp. serovars in animals.

CONCLUSION

High *Leptospira* spp. infection rate was found in cattle bred in the indigenous reserves of Barra do Corda, Maranhão State. The serovars most often found in the current study were Shermani, Guaricura, Sentot, Djasiman, Patoc, Hardjo C.T.G, Hardjo Bovis, Hardjo, Hardjo Prajitno and Wolffi. Antibodies against the first five serovars pointed towards the key role played by the local wildlife in the infection process. The other serovars evidenced the significant role played by cattle in the spread and maintenance of leptospirosis. With respect to the epidemiology of the infection in the studied area, variables such as reproductive clinical signs, environmental factors, local infrastructure and sanitary management aspects should be taken into consideration to help preventing leptospirosis.

ACKNOWLEDGEMENTS

The Foundation for Scientific Research and Development of Maranhão (FAPEMA) and State University of Maranhão (UEMA) for making this study possible.

REFERENCES

ARAÚJO, V. E. M. et al. Frequência de aglutininas anti-*Leptospira interrogans* em soros sanguíneos de bovinos, em Minas Gerais, de 1980 a 2002. **Arquivo Brasileiro de Medicina Veterinaria e Zootecnia**, v. 57, p. 430-435, 2005.

BRASIL. Ministério da Saúde. Centro Nacional de Epidemiologia. **Manual de leptospirose**. 2ª ed. Centro Nacional de Epidemiologia. Coordenação de Controle de Zoonoses e Animais Peçonhentos, Brasília, 1995. 98p.

BRASIL. Ministério da Saúde. Leptospisose. In: ____. **Guia de vigilância epidemiológica**. 6. ed. Brasília: Secretaria de Vigilância em Saúde, 2005. 816p.

CAMPOS Jr., A. C. P. D. et al. Prevalência de anticorpos anti-*Leptospira* em machos bovinos na microrregião de Goiânia. **Ciência Animal Brasileira**, v. 7, p. 439-446, 2006.

CARVALHO, O. S. et al. Occurrence of *Brucella abortus*, *Leptospira interrogans* and bovine herpesvirus type 1 in buffalo (*Bubalus bubalis*) herd under extensive breeding system. **African Journal Microbiology Research**, v. 9, n. 9, p. 598-603, 2015.

CASTRO, V. et al. Soroprevalência da leptospirose em fêmeas bovinas em idade reprodutiva no Estado de São Paulo, Brasil. **Arquivos do Instituto Biológico**, v. 75, n. 1, p. 3-11, 2008.

COELHO, E. L. M. et al. Prevalência de leptospirose em fêmeas bovinas abatidas em frigoríficos no município de São Luís, MA. **Revista Brasileira de Medicina Veterinaria**, v. 36, n. 2, p. 111-115, 2014.

CUBAS, Z. S.; SILVA, J. C. R.; CATÃO-DIAS, J. L. **Tratado de animais selvagens: medicina veterinária**. 2. ed. São Paulo: Roca, 2014. 2512 p.

GARBA, B et al. Major epidemiological factors associated with leptospirosis in Malaysia. **Acta Tropica**, v. 178, n. 242-247, 2018.

GENOVÉZ, M. E. Leptospirose: uma doença de ocorrência além da época das chuvas. **Biológico**, v. 71, n. 1, p. 1-3, 2009.

HASHIMOTO, V. Y. et al. Prevalência e fatores de risco associados à *Leptospira* spp. em rebanhos bovinos da região,centro-sul do estado do Paraná. **Pesquisa Veterinária Brasileira**, v. 32, p. 99-105, 2012.

JORGE, S. et al. Human and animal leptospirosis in Southern Brazil: A five-year retrospective study. **Travel Medicine and Infectious Disease**, v. 18, p. 46-52, 2017.

KIRKBRIDE, C. A. Leptospiral abortion. In: _____. **Laboratory Diagnosis of Livestock abortion**. 3rd ed. State University Press, Ames, 1990. p. 59-65.

MARANHÃO. Agência Estadual de Defesa Agropecuária do Estado do Maranhão. Setor de Epidemiologia e Estatística, Coordenadoria de Defesa Animal. **Banco de dados**. 2016.

MARIANO, F. da S. **Estudo preliminar da pecuária bovina em terra indígena situada na região de savana**. 2011. 39 p. Monografia (Graduação em Zootecnia). Universidade Federal de Roraima, Boa Vista, 2011

NAGEL, A. et a.. Bovine macrophages responses to the infection with virulent and attenuated Leptospira interrogans serovar Pomona. **Veterinary Microbiology**, v. 233, p. 124-132, 2019

NOORDHUIZEN, J. P. T. M. et al. **Application of Quantitative Methods in Veterinary Epidemiology**. Wageningen Pres, Wageningen, 1997. 445p.

OIE. Wold Organisation For Animal Healt. **Leptospirosis**. 2010. Disponivel em: <http://www.oie.int/eng/normes/mmanual/a_00043.htm> Acesso em: 21 jun. 2017.

OLIVEIRA, F. C. S. et al. Soroprevalência de leptospirose em fêmeas bovinas em idade reprodutiva no estado da Bahia. **Arquivos do Instituto Biológico**, v. 76, p. 539-546, 2009.

PAIXÃO, A. P. et al. *Leptospira* spp. em bovinos leiteiros do estado do Maranhão, Brasil: frequência, fatores de risco e mapeamento de rebanhos reagentes. **Arquivos do Instituto Biológico**, v. 83, n. 1-12, e1022014, 2016.

PELLEGRIN, A. O. et al. **Prevalência da leptospirose em bovinos do Pantanal Mato-Grossense**. Comunicado Técnico, Embrapa Pantanal, Corumbá, 1999. 9p.

PEREIRA, E. S. Centro de Informações Diocese de Roraima. **Relatório Estatístico/1990.** Boa Vista, 1990. 10 p.

PHILIP, S. S. Spirochetal infections. In: MC Phee, S. J. and Papadakis, M. A. (Eds.). **Current Medical Diagnosis & Treatment**. New York: McGraw-Hill, 2011. p.1414-1415.

SARMENTO, A. M. C. et al. Emprego de estirpes *Leptospira* spp. isoladas no Brasil na microtécnica de soroaglutinação microscópica aplicada ao diagnóstico de leptospirose em rebanhos bovinos de oito estados brasileiros. **Pesquisa Veterinária Brasleira**, v. 32, n. 7, p. 601-606, 2012.

SPEELMAN, P.; HARTSKEERL, R. A. Leptospirosis. In: Kasper, D., Fauci, A. S., Hauser, S., Longo, D., Jameson, J. L.; Loscalzo, J. **Harrison's principles of internal medicine**. New York: McGraw-Hill, 2008. p.1048-1051.

SILVA, F. J. et al. Anticorpos contra *Leptospira* spp. emanimais domésticos e silvestres presentes no Campus Universitário da FCAV, UNESP, Jaboticabal/SP. **ARS Veterinária**, v. 26, n. 1, p. 17-25, 2010.

SILVA, F. J. Prevalência e fatores de risco de leptospirose bovina no estado do Maranhão. **Pesquisa Veterinária Brasileira**, v. 32, n. 4, p.303-312, 2012.

TEIXEISRA, A. F. et al. Adjuvanted leptospiral vaccines: Challenges and future development of new leptospirosis vaccines. **Vaccine**, v. 8, p. S0264-410X(19)30734-0, 2019.

VASCONCELLOS, S. A. Leptospirose bovina. Níveis de ocorrência e sorotipos predominantes em rebanhos dos Estados de Minas Gerais, São Paulo, Rio de Janeiro, Paraná, Rio Grande do Sul e Mato Grosso do Sul. Período de janeiro a abril de 1996. **Arquivos do Instituto Biológico**, v. 64, n. 2, p. 7-15, 1997.

VIEIRA, A. S.; LILENBAUM, W. Leptospirosis on captive wild animals in Latin America. **Research in Veterinary Science**, v. 115, p. 496-500, 2017.