Epidemiological aspects of leptospirosis in cattle herds from indigenous reserves
Aspectos epidemiológicos da leptospirose em rebanhos bovinos de reservas indígenas

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ABSTRACT

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 e Icterohemorrhagiae. 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.

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 região 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 Icterohemorrhagiae. Das variáveis epidemiológicas, a não destinação correta aos fetos abortados e restos placentários, ocorrência de abortamento nos últimos 12 meses, concentração de...
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; TEIXEIRA 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 Leptospirosa 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.

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

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          |
| Autumnalis      | Autumnalis                     |
| Ballum          | Butembo; Castellonis           |
| Batavia         | Bataviae                       |
| Canicola        | Canicola                       |
| Calledoni       |                                 |
| Cynopteri       |                                 |
| Grippotyphosa   |                                 |
| Hebdomadis      | Hebdomadis                     |
| Icterohaemorragie | Copenhageniens;                |
|                 | Icterohaemorrhagiae            |
| Javanica        | Javanica                       |
| Panama          | Panama                         |
| Pomona          | Pomona                         |
| Pyrogenes       |                                 |
|                 | Pyrogenes                      |
|                 | Hardjo; Wolfi, Guaricura,      |
|                 | Hardjo Bovis, Hardjo           |
| Serjroe         |                                 |
|                 | Prajitno, Hardjo C.T.G,        |
|                 | Hardjo Miniswajizak            |
| Shermani        | Shermani                       |
| Terassovi       | Terassovi                      |
| Andamana        | Andamana                       |
| Semaranga       | Patoc                          |
| Djasiman        |                                 |
|                 | Sentot, Djasiman                |

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 | Herds/Indigenous Reserve | Cattle |
|---------------|--------------------------|--------|
|               | N | Reactive | % | N | Reactive | % |
| 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 | 47 | 78.33 |
| Total         | 05 | 05 | 100 | 300 | 240 | 80.00 |

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%), Wolfi (68/300; 22.61%), Hebdomadis (53/300; 17.67%), Adamanda (43/300; 14.33%) and Icterohaemorrhagiae (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 Grajau 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 Grajau 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%         |
| Guariacura    | 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%         |
| Wolfi         | 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%          |
| Hadjo Miniswajzak | 16   | 5.33%          |
| Copenhageni   | 16       | 5.33%          |
| Nupezo        | 10       | 3.33%          |
| Cynopect      | 09       | 3.00%          |
| Javanica      | 08       | 2.67%          |
| Pyrogenes     | 08       | 2.67%          |
| Panamá        | 07       | 2.33%          |
| Pomoa         | 06       | 2.00%          |
| Castelonis    | 05       | 1.67%          |
| Batavae       | 02       | 0.67%          |
| Brasilvila    | 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 | Itaiapava do Grajau | Jenipapo Vieiras dos | total |
|---------------|------------------|-------|----------------|--------|---------------------|----------------------|-------|
|               | React. | %     | 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% |
| Guariacura    | 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  | 00.00%|
| 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  | 46.3% | 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% |
| Wolfi         | 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%|
| Canicola      | 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  | 08.3% |
| 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 Miniswajzak | 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%|
| Cynopect      | 01/60   | 01.67%| 05/60  | 08.3% | 03/60  | 05.00%| 00/60  | 00.00%| 00/60  | 00.00%|
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 pacaa*) 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, Hebdomensis 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; OELHO 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 Wolffii (22.00%), Patoc (12.42%), Shermani (8.85%), Grippotyphosa (8.21%) and Hebdomadis (7.35%).

Hardjo C.T.G (29.67%), Hardjo Bovis (27.67%), Hardjo (26.67%) and Hardjo Prajitno (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, Wolff, Guaricura, Hardjo Bovis, Hardjo Prajitno, Hardjo C.T.G., Hardjo Miniswajizak), as reported by Samento et al. (2012). However, definitive confirmation depends on further investigations based on sample isolation and typification.

The Wolffii 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 Wolffii, 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 Wolfii 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 Wolfii 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 Leptosira spp. serovars in animals.

**CONCLUSION**

High Leptosira 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 Shermanni, Guaricura, Sentot, Djasiman, Patoc, Hardjo C.T.G, Hardjo Bovis, Hardjo, Hardjo Prijatin and Wolfi. 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 sanitation management aspects should be taken into consideration to help preventing leptospirosis.

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