



Original Article

***Leptospira* spp. in sheep of the state of Maranhao, Brazil: frequency, risk factors and foci mapping**

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ARTICLE INFO

Article history

Received 10 November 2017

Received in revised form 22 January 2018

Accepted 14 February 2018

Keywords:

Sheep

Leptospirosis

Microscopic sero - agglutination

Maranhao

ABSTRACT

The objective of this research was to estimate the frequency, risk factors and mapping of outbreaks in ovine flocks in the State of Maranhao, Brazil, related to *Leptospira* spp. A total of 575 samples of blood serum from sheep were collected, from 116 flocks, and 20 municipalities. An epidemiological questionnaire was applied to verify the association of risk factors. Microscopic Agglutination Test (MAT) was used for 24 serovars of *Leptospira* spp. 100% of the flocks (116) and 94.43% of animals (543/575) were reagents for at least one of the 24 serovars tested. The most frequent serovars were *Sentot* (236, 41.04%), *Hadjo* (197, 34.26%), *Adamana* (178; 30.95%), *Pomona* (158, 27.47%) and *Autumnallis* (148, 25.73%). The presence of rodents, felines, goats, pigs and wild animals had a statistically significant association ($p < 0.05$) with the occurrence of *Leptospira* spp. It was concluded that the frequency of *Leptospira* spp. was high in sheep from the State of Maranhao. However, all municipalities had infected animals, therefore, it is considered necessary to implement sanitary measures for the control and prevention of this disease in ovine flocks in the state of Maranhao.

INTRODUCTION

Leptospirosis is an infectious, bacterial disease classified as anthroponosis. It was considered to have a high prevalence in herds, especially in Latin American and Central American countries, causing serious economic losses to livestock (ESCÓCIO et al., 2010; PAIXÃO et al., 2016). Its etiology is a bacterium of the genus *Leptospira*, with special emphasis on the species *L. Interrogans* and *L. Biflexa* (MACHADO, 2016). In sheep, the clinical manifestation may present jaundice, hemoglobinuria, bloody mastitis, hemorrhage, return to estrus and abortions (HIGINO, 2010).

According to ESCÓCIO et al., 2010, any serovar of *Leptospira* spp. can infect domestic animals, however, it will depend on which serovars are predominant, climatic and environmental conditions, as well as the type of management used in that region or property.

It is important to highlight the relevance of the spatial study as an alternative for the creation of control strategies and epidemiological surveillance of leptospirosis. This procedure can be performed with the use of specific software that allows the visualization, exploration, and modeling of geo-referenced data (MACHADO et al., 2016).

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Considering the importance of sheep farming as a source of income generation in family farming, motivation for setting the man in rural area, allied, in exchange for the lack of data for a better understanding of its productive chain from the sanitary point of view; an evaluation of the sheep flock of the state of Maranhao is necessary. Thus, this research was developed with the objective of verifying the frequency, risk factors, and serovars, as well as mapping foci of *Leptospira* spp. in sheep flocks of Maranhao state.

MATERIAL AND METHODS

575 samples of sheep blood serum were collected from 116 flocks distributed in the municipalities that make up the regions of Chapadinha and Itapecuru Mirim, untangling: Agua Doce, Anapurus, Araiases, Brejo, Buriti, Chapadinha, Magalhaes of Almeida, Paulino Neves, Santa Quitéria, Sao Bernardo, Tutoia, Anajatuba, Cantanhede, Itapecuru Mirim, Matoes do Norte, Miranda do Norte, Pirapemas, Presidente Vargas, Santa Rita and Vargem Grande. The CALLEGARI-JACQUES (2003) formula was used, with a sampling error of 8%, following the principle of population proportionality of animals and herds for each municipality. Flocks were chosen randomly, using data provided by the Department of Epidemiology of the State Agricultural and Livestock Agency of the state of Maranhao (AGED - MA). The animals were randomly chosen regardless of age, race and gender.

Blood was collected by jugular vein puncture using disposable needles (25 x 8mm) in 8ml tubes, vacuum, and transported to the Laboratory of Infectious Diseases of the State University of Maranhao. Subsequently, it was centrifuged at 2500 G / 5min. Samples were identified with the number of the animal and property, maintained at - 20 ° C. For each flock, an epidemiological questionnaire was used to obtain information to evaluate possible risk factors associated with Leptospirosis.

For the geo-referencing of foci, global positioning satellite navigation (GPS) was used. Each property is located at a point in space and identified by the AGED-MA (2012) database. GPS Track Maker® software version 13.0 was used to obtain the maps.

The diagnosis of leptospirosis was performed using the Microscopic Agglutination Test (MAT) technique, according to GALTON et al. (1965) and COLE et al. (1973). Antigens of 24 serovars of the *Leptospira* spp. some of which are: *Andamana*, *Guaricura*, *Shermani*,

Cynopteri, *Butembo*, *Panama*, *Hardjobovis*, *Castelonis*, *Whitcombi*, *Tarassovi*, *Javanica*, *Australis*, *Autumnalis*, *Bataviae*, *Bratislava*, *Canicola*, *Copenhagenihageni*, *Grippothyphosa*, *Hebdomadis*, *Icterohaemorrhagiae*, *Pomona*, *Sentot*, *Wolffi*, and *Pyrogenes*. Reagent samples were considered when anti-*Leptospiraspp* agglutinins were present in a proportion equal to or greater than 50% of Leptospires. After confirmation, the reagent sera were evaluated according to the degree of agglutination, respecting the criteria described: 1 + (less than 50% agglutinated Leptospires), 2 + (between 51% and 74% agglutination) and 3+ (75% up to 100% agglutination). Samples with titers ranging from 100 to 800 were reagent.

The antigen was made with live cultures of *Leptospira interrogans*, grown on FLETCHER (1928) and EMJH (DIFCO®) semi-solid media supplemented with 10% sterile rabbit serum. These media were distributed in 48 test tubes, 24 tubes for each medium. They were incubated in a bacteriological oven at 28 to 30 ° C for 7 to 14 days. Weekly tests were performed on new tubes for each culture medium.

All flocks were submitted to an epidemiological survey to evaluate possible risk factors, such as flooded areas; presence of rodents, felines, goats and pigs; veterinary assistance, extensive breeding; presence of wild animals; history of abortion; natural breeding; quarantine; rental of grass; fate of aborted fetuses; vaccination against leptospirosis.

Frequency was calculated by dividing the number of reactive animals by the number of animals in the sample employing descriptive statistical analysis by using absolute and relative values. To verify the association between the reactive herds and risk factors analyzed, statistical analysis was performed using the Chi-Square test of independence. The level of significance used in the statistical test decision was 5% (0.05) and Confidence Intervals of 95%. The program used for analysis was Instat 2.0 version 2013 and Ep Info 3.43 version 2007.

This study was developed after submission to the Animal Ethics and Experimentation Committee under Protocol No. 041/2014 on 12/12/2014, from March 2014 to January 2015.

RESULTS

According to the data analyzed, 100% of the herds (116) and 94.43% of animals (543/575) were reactive for at least one of the 24 serovars tested (Table 1).

Table 1 – Frequency of sheep and herds reactive to *Leptospira* spp. in the municipalities that belong to the Regional Units of Chapadinha and Itapecuru Mirim, MA.

Regional Units	Nº Flocks	Rebanhos		Animais	
		Reactive	Non-reactive	Reactive	Non-reactive
Chapadinha	61	61	0	268	32
ItapecuruMirim	55	55	0	275	0
Total	116	116(100%)	0	543(94.43%)	32(5.57%)

The serotype frequency results are described in Table 2.

Table 2 – Frequency of anti-*Leptospiraspp*serovars, diagnosed in sheep in the municipalities belonging to the Regional Units of Chapadinha and ItapecuruMirim, MA, 2015.

Serovars	Nº animals	ReactiveNo./%
<i>Australlis</i>	575	26/ 4.52
<i>Brastilava</i>	575	53/ 9.25
<i>Autumnallis</i>	575	148/ 25.73
<i>Butembo</i>	575	96/ 16.69
<i>Castelone</i>	575	71/ 12.34
<i>Batavae</i>	575	98/17.04
<i>Canicola</i>	575	89/ 15.47
<i>Sentot</i>	575	236/ 41.04
<i>Whitcombi</i>	575	52/ 9.04
<i>Cynopte</i>	575	55/ 9.56
<i>Grippytyphosa</i>	575	133/ 23.13
<i>Hebdomadis</i>	575	143/ 24.86
<i>Compen</i>	575	93/ 16.17
<i>Ictero</i>	575	44/ 7.65
<i>Javanica</i>	575	69/ 12.00
<i>Panamá</i>	575	42/ 7.30
<i>Pomona</i>	575	158/ 27.47
<i>Pyrogenes</i>	575	83/ 14.43
<i>Hadjo</i>	575	197/ 34.26
<i>Wolf</i>	575	130/ 22.60
<i>Shermani</i>	575	108/ 18.78
<i>Taransovi</i>	575	43/ 7.47
<i>Adamana</i>	575	178/ 30.95
<i>Guaricura</i>	575	95/ 16.52

As for the number of foci for leptospirosis, 100% (n = 116) reacted to this disease. Figures 1 and 2 show the spatial study, where the dots represent the reactive herds. In municipalities with higher sampling, it is possible to observe the concentration of foci, as well as spatial vacuums that should be addressed in future studies for this disease (Figures 1 and 2).

The analysis of the risk factors indicated that the presence of rodents, felines, goats, pigs and wild animals had statistical significance ($p < 0.05$) and may be related to *Leptospira* spp. infection. However, the risk factors for flooded areas, history of abortion and vaccination against leptospirosis, despite not being statistically significant, presented odds ratio above one, that is, the

probability of these factors being associated with *Leptospira* spp. is considerable (Table 3).

DISCUSSION

The results of this research evidenced a high frequency of flocks, as well as, from animals to agglutinins of *Leptospiraspp*. Studies carried out in several Brazilian states demonstrated how prevalent this disease is in herds and animals. It was observed that in the case of Sao Paulo, 100% (FAVERO et al., 2002) and 51% (GENOVEZ et al., 2011), in Rio Grande do Sul 34% (HERRMANN et al., 2004), Piaui 28% (CARVALHO et al. 2011), Parana 100% (HASHIMOTO et al. 2010), Rio Grande do Norte 14% in goats (ARAÚJO NETO et al. 2010), Minas Gerais 22% (SALABERRY et al. 2011), Paraiba 7.5% (HIGINO et al. 2010). Pernambuco 19.5% (MACHADO et al., 2016) and in Maranhao in cattle Paixão et al. (2016) found 100% of bovine reagents.

The variation of the prevalence results for *Leptospira* spp. with sheep in Brazil, may be associated with bioclimatic factors that may directly interfere with the infection by this agent. In this way, the temperature, humidity, rainfall indexes, regional topography, access to a water source in the properties, as well as the presence of wild and domestic reservoirs, are highlighted.

The high frequency of the serovar *Sentot* may be associated with the infection in humans since this serovar is related to the clinical disease in human species. Values lower than this research were described by ALVES et al. (2012) which found 17.39% in the Brazilian semi-arid regions. This serovar was diagnosed in domestic animals by HERRMANN et al. (2004), with the prevalence of 16.8%. The result of this research makes evident the need to carry out a work with breeders who were collaborators, in order to identify the occurrence of *Leptospira* spp. in humans.

The serovar *Hardjo* is responsible for reproductive losses in cattle and some authors report that the majority of cases of abortion in sheep are linked to this serovar (LILENBAUM et al., 2008). According to Marinho et al. (2012), the presence of this serovar may be related to the management of sheep in conjunction with cattle.

Figure 1 – Map showing the foci sheep flocks for *Leptospira* spp. in the regional of Chapadinha, Maranhao, Brazil, 2015.

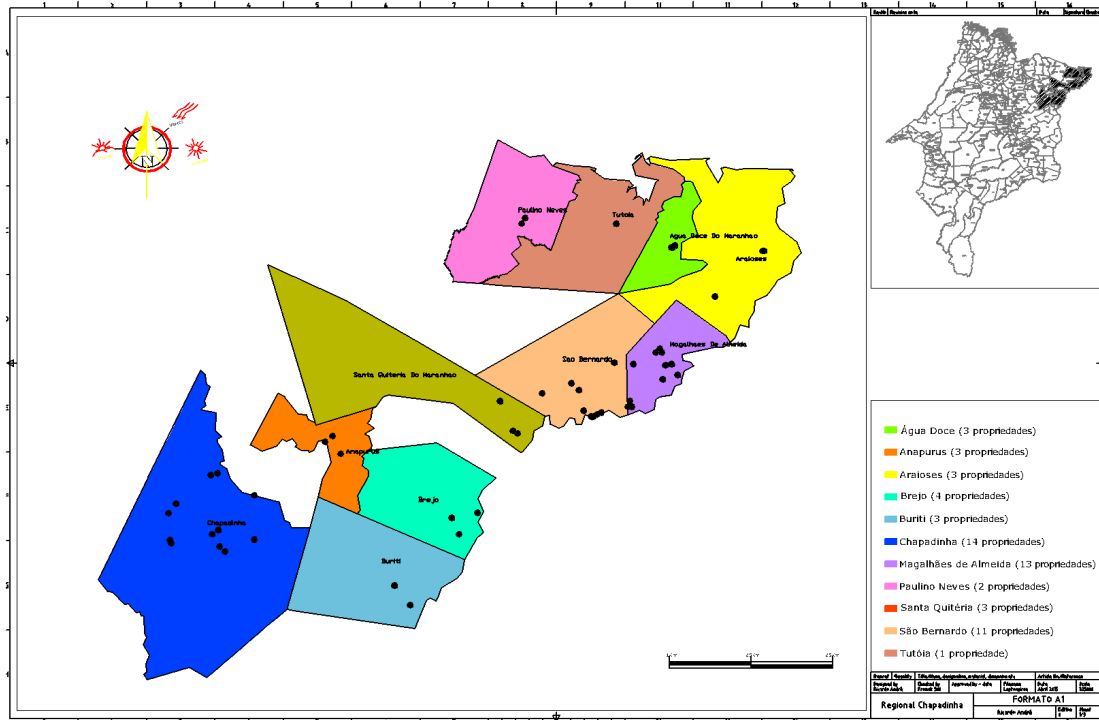
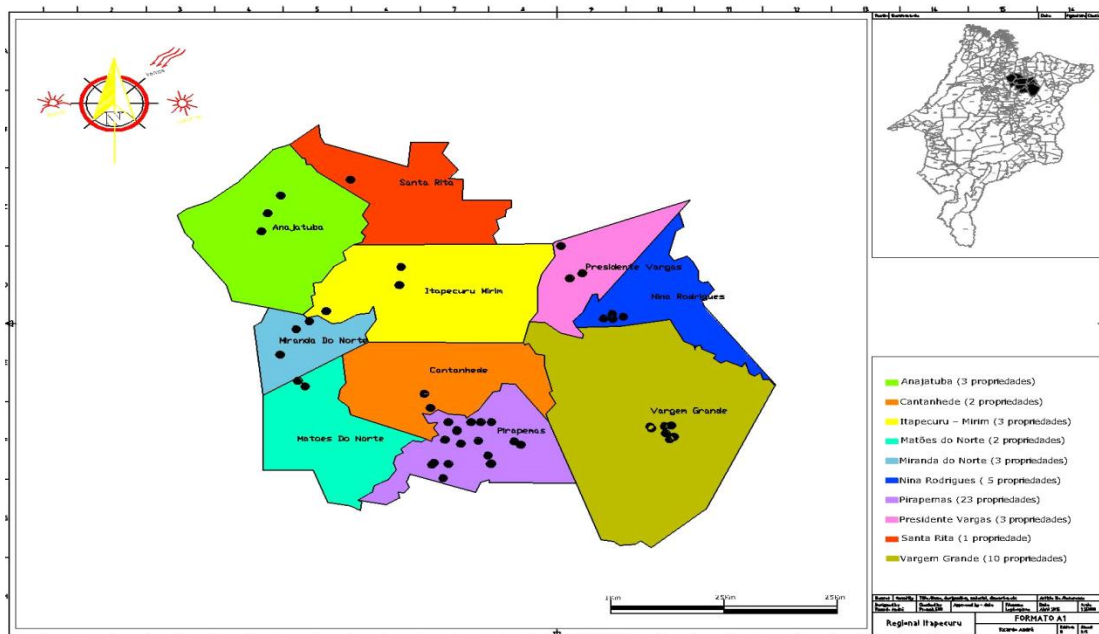


Figure 2 – Map showing foci sheep flocks for *Leptospira* spp. at the regional of ItapecuruMirim, Maranhao, Brazil.



As for serovar *Andamana*, AGUIAR et al. (2010) reported that this serovar is an important serological marker, since it may present a cross-reaction with other pathogenic serovars.

The serovar *Pomona* has been described in other studies as the main cause of clinical leptospirosis and abortion cases for sheep (CARVALHO et al., 2011; LANGONI et al.,

1995). However, in this research, few reports have been described on these aspects.

Serovar *Autumnallis* presented a lower frequency among the five most diagnosed in MAT. However, ALVES et al. (2012) found 49.3% and ARAÚJO NETO et al. (2010) 73.6% in goats. This serovar has as main reservoir the rodents, which shows the importance of these animals in the transmission of *Leptospira* spp.

Table 3 – Univariate analysis of risk factors associated with *Leptospira* spp. infection in ovine herds of the regional ones of Chapadinha and Itapecuru Mirim, Maranhao, Brazil, 2015.

VARIANTS	Description	Univariate analysis			Value p*
		Reactive	Non-reactive	OR (IC 95%)	
Flooded areas	Yes	285	15	1.41	0.3783
	No	256	24	0.70-2.83	
Presence of rodents, felines, goats and pigs	Yes	315	25	0.44	0.0467*
	No	227	13	0.19-1.00	
Veterinary assistance	Yes	112	11	0.57	0.1396
	No	428	29	0.27-1.20	
Extensive breeding	Yes	379	35	0.47	0.1273
	No	160	6	0.19-1.16	
Presence of wild animals	Yes	61	9	23.50	0.0001*
	No	478	32	10.56-52.33	
History of abortion	Yes	287	33	1.31	0.4415
	No	242	18	0.70-2.43	
Natural breeding	Yes	413	37	0.40	0.1012
	No	126	4	0.14-1.18	
Quarantine	Yes	120	10	0,77	0.5422
	No	418	32	0.36-1.64	
Rental of grass	Yes	247	18	0.79	0.6005
	No	293	22	0.40-1.57	
Fate of aborted fetuses	Bury	146	14	0.61	0.1841
	Do nothing	392	28	0.30-1.22	
Vaccination against leptospirosis	Yes	124	6	1.44	0.5341
	No	416	34	0.58-3.55	

The identification of outbreaks in sheep flocks for *Leptospira* spp. showed that there is a wide distribution of reactive animals in all the municipalities that make up the regions of Chapadinha and Itapecuru Mirim. This wide distribution may be associated with the commercialization of infected animals from these municipalities, as well as the absence of almost all sanitary control of the flocks for leptospirosis. For, according to data from the World Health Organization (2014), transit and acquisition of animals are considered as permanent sources of infection to those susceptible, as well as, exhibitions and auctions, which are used in these regions as marketing tools.

Regarding the risk factors associated with *Leptospira* spp infection in reactive sheep, the presence of rodents, felines, goats, pigs and wild animals with significant results in this research are conspicuous. While Salaberry et al. (2011) described as factors associated with the risk of infection by *Leptospira* spp., sex, race and the presence of rodents. According to Genovez et al. (2011) conjunction involved in the breeding of production animals, especially sheep, goats, and cattle, may be associated with the transmission of diseases, such as leptospirosis.

Therefore, it is necessary to adopt measures to minimize the risk of infection by *Leptospira* spp., Such as the disinfection of facilities, the use of adequate management for each breeding in the property, as well as the systematization of prophylactic control measures,

such as quarantine of newly acquired animals and periodic vaccination of herds.

CONCLUSIONS

According to the results of this study, it was concluded that the frequency of *Leptospira* spp was high in sheep from the state of Maranhao. *Sentot*, *Hardjo*, *Andamana*, *Pomona* and *Autumnalis* serovars were the most frequent, and the risk factors that may be associated with the infection for leptospirosis were; Presence of rodents, felines, goats, swine and wild animals. All municipalities had infected animals. Consequently, it is necessary to adopt sanitary measures for the control and prevention of this disease in sheep herds of the state of Maranhao.

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