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Epidemiologic analysis of *Leptospira* spp. infection among sheep in Pernambuco state, Brazil

Análise epidemiológica da infecção por **Leptospira** *spp. em ovinos no estado de Pernambuco, Brasil*

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ABSTRACT: This study aimed to analyze epidemiological aspects and spatial characteristics of *Lesptospira* spp. infection among sheep in Pernambuco state, Brazil. A total of 426 samples were collected from unvaccinated animals aged 1 year or older, and submitted to Microscopic Agglutination Test (MAT). To study the risk factors, a standardized investigative survey of sheep farmers, with objective questions, was conducted to establish the general characteristics of the farm property production, reproductive management, and health plan. From 426 samples analyzed, 83 (19.5%; CI 15.9 – 23.6%) were positive; among them, the most prevalent serovar was Autumnalis (32.4%). Significant differences were observed between gender (OR = 0.38; CI 0.14 – 0.99), production system (OR = 2.03; CI 1.07 – 3.84), types of herd (OR = 2.28; CI 1.39 – 3.72), absence of rodents to the grain storage (OR = 0.55; CI 0.34 – 0.91), and restriction to surface waters (OR = 0.60; CI 0.37 – 0.99). *Leptospira* spp. infection is disseminated in sheep herds, and multiple factors can influence its occurrence. Health education programs and worker training in prevention and control of leptospirosis and other infectious diseases may be useful to reduce infection rates and economic losses caused by this disease.

KEYWORDS: epidemiology; leptospirosis; sheep industry.

RESUMO: Objetivou-se com este estudo analisar os aspectos epidemiológicos e caracterizar espacialmente a infecção por *Leptospira* spp. em ovinos no estado de Pernambuco, Brasil. Para compor a amostragem do estudo, foram colhidas 426 amostras de animais com idade igual ou superior a um ano, não vacinados, e submetidas à reação de Soroaglutinação Microscópica (SAM). Para o estudo dos fatores de risco, aplicou-se um questionário investigativo padronizado, constituído por perguntas objetivas ao criador, referentes às características gerais da propriedade, ao manejo produtivo, reprodutivo e sanitário. Das 426 amostras analisadas, 83 (19,5%; IC 15,9 – 23,6%) foram positivas; entre elas, o sorovar de maior prevalência foi o Autumnalis (32,4%). Foram observadas diferenças significativas entre sexo (OR = 0,38; I.C. 0,14 – 0,99), sistema de criação (OR = 2,03; I.C. 1,07 – 3,84), tipo de rebanho (OR = 2,28; I.C. 1,39 – 3,72), ausência do acesso de ratos a ração (OR = 0,55; I.C. 0,34 – 0,91) e restrição a águas de superfície (OR = 0,60; I.C. 0,37 – 0,99). A infecção por *Leptospira* spp. está distribuída nos rebanhos ovinos e são múltiplos os fatores que podem influenciar sua ocorrência. A implantação de programas de educação em saúde e o treinamento da mão de obra em métodos de prevenção e controle da leptospirose e outras doenças infecciosas podem ser úteis na redução dos casos de infecção e na diminuição das perdas econômicas causadas por esta enfermidade.

PALAVRAS-CHAVE: epidemiologia; leptospirose; ovinocultura.

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INTRODUCTION

Leptospirosis is an infectious disease, which determines economic losses in livestock, mainly characterized by reducing the reproductive performance in herds (Aguiar et al., 2010).

Leptospirosis in small ruminants is usually asymptomatic, which ensures the permanence of carrier animals spreading the agent in flocks, and increases the risks of infection (Herrmann et al., 2004; Araújo Neto et al., 2010).

Studies conducted by different microscopic serum agglutination techniques in some States of Brazil have detected anti-*Leptospira* antibody in sheep, with wide variation of anti-*Leptospira* prevalence and serovars (Langoni et al., 1995; Caldas et al., 1995; 1996; Mota et al., 1999; Herrmann et al., 2004; Aguiar et al., 2010; Hashimoto et al., 2010; Higino et al., 2010, Carvalho et al., 2011; Salaberry et al., 2011; Alves et al., 2012; Martins et al., 2012).

Leptospira spp. infection in sheep has been related to environmental factors such as temperature, moisture, water and soil pH, and presence of organic matter (Escócio et al., 2010) and/or management-related factors, such as mixed species grazing (Langoni et al., 1995), semi-intensive system (Mota et al., 1999), presence of carriers, not carrying out quarantine to all new arrivals to the flock (Salaberry et al., 2011), and animal participation in events (Alves et al., 2012).

The spatial analysis, which involves the use of environmental software, allows the visualization, exploration, and modeling of georeferenced data (Gatrell; Bailey, 1996); it also supports the creation of plans for preventive actions and health surveillance (Fonzar; Langoni, 2012).

The sheep industry is an important production chain in the State of Pernambuco, which generates income for small farmers; however, because of the lack of studies on the occurrence of infectious diseases, it is worth highlighting that highlight leptospirosis that may cause direct and indirect losses in sheep flocks, as well as risks to public health. The present study aimed to examine epidemiological aspects and to spatially characterize the distribution of the *Leptospira* spp. infection among sheep in Pernambuco State, Brazil.

MATERIAL AND METHODS

This study was performed in accordance with the ethical principles of animal experimentation established by the Animal Use Ethics Committee of Universidade Federal de Pernambuco, as listed under protocol no. 018/2012, issued by the president of the committee on July 13, 2012.

The cross-sectional study was conducted on 14 properties located in 11 municipalities of mesoregions Agreste – Vale do Ipanema (Águas Belas, Itaíba e Pedra), semiarid of Pernambuco-Itaparica (Carnaubeira da Penha, Floresta, Itacuruba, Tacaratu), and semiarid of Moxotó (Arcoverde, Custódia, Ibimirim, Sertânia).

A total of 426 samples were collected, providing a safety margin, considering a calculation of prevalence of *Leptospira* spp. infection in sheep for the sample of 33.7% (Borba, 2004), 95% confidence level and 5% standard error (Thrusfield, 2005), whose minimum sampling is 344 animals.

Before the collection of biological samples, a standardized investigative survey was applied to sheep farmers, containing objective questions to establish general characteristics of the farm property production, reproductive management, and health plan, as well as to study the risk factors.

Diagnosis of serum samples was tested by Microscopic Agglutination Test (MAT; Galton et al., 1965; Cole et al., 1973), using, as antigens, 23 alive leptospiras sorovars, such as Andamana, Australis, Autumnalis, Bataviae, Bratislava, Butembo, Canicola, Castellonis, Copenhageni, Cynopteri, Hardjo, Hebdomadis, Grippotyphosa, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Sentot, Shermani, Tarassovi, Whitcombi, and Wolffi, grown on Ellinghausen-McCullough-Johnson-Harris (EMJH) medium, transferred weekly, used after the fourth day in BOD incubator at 28°C, contamination free, and self-agglutination free. Those sera showing at least 50% of agglutination of leptospires in the microscopic field from the 1:100 dilution were considered positive (Galton et al., 1965; Cole et al., 1973; Silva et al., 2012).

To identify risk factors associated with infection by *Leptospira* spp., an univariate analysis was conducted according to variables of interest through Pearson's chi-square test or Fisher's exact test, when necessary, and multivariate analysis by logistic regression model, considering the serological status of the animal (positive or negative) as a dependent variable. Those variables that showed statistical significance < 0.20 were considered either independent or explanatory. The probability was stipulated to prevent possible risk factors that were not excluded from the analysis. EpiInfo 3.5.2 software was used in statistical calculations.

Prevalence was calculated using Epiinfo™ software (version 7), considering a 95% confidence interval. The properties were considered as null prevalence if there were no reagent animals; low prevalence, if the number of reagents was below 25%; average prevalence, between 25% and 50%, and high if the number of reagents was higher than 50%. The infective serovar for the animals was determined based on the higher titer. Those cases in which there was serological coagglutination at the same titer were not considered for analysis of the serovar prevalence.

For verification of the spatial distribution of infection in the Agreste and Sertão regions of Pernambuco, the plane coordinates obtained by georeferencing for every property by Global Positioning System (GPS) were used, which were set up to provide the positions on the coordinate plane in the Universal

Transverse Mercator (UTM) projection, the South American Datum of 1969 system (SAD-69), corresponding to the coordinate system of Cartographic Base from study regions. For the mapping and the distribution of serovars, georeferenced data were added on the Terra View 3.1.3 software (Brazil, 2006).

RESULTS

Prevalence of anti-Leptospira antibody in sheep was 19.5% (83 of 426 properties; CI 15.9 – 23.6). In 92.8% of properties, sites of infection were found (13 of 14 properties). Analyzing the prevalence of infection by property, 3 (21.4%) presented average prevalence, 10 (71.4%) presented low prevalence, and 1 (7.2%) presented prevalence zero (Figure 1).

The highest occurrence was the serovar Autumnalis (32.4%), followed by Icterohaemorrhagiae (14.0%), and Castellonis (9.8%). However, the main serovar spread in the studied area was Icterohaemorrhagiae (Figure 2). In 12 samples, it was not possible to identify the infective serovar, as result of two or more serovar coagglutination reaction. Serovars identification and their respective titer are presented in Table 1.

Statistically significant variables on the univariate analysis of risk factors were: region ($p = 0.003$), breeding system ($p = 0.016$), gender ($p = 0.0026$), access to water ($p = 0.030$), type of herd ($p < 0.001$), the presence of dogs on the property ($p = 0.047$), and the absence of rodents on the grain storage ($p = 0.013$). According to logistic regression (Table 2), the following have been identified:

1. Risk factors: breeding system (OR = 2.03; $p = 0.029$) and type of herd (OR = 2.28; $p = 0.001$); and
2. Protective factors: absence of rodents to the grain storage (OR = 0.55; $p = 0.020$), restricted access to surface water (OR = 0.60; $p = 0.047$), and gender (OR = 0.38; $p = 0.049$).

No association was identified between occurrence of reproductive disorders (abortions, weak Cubs, retained placenta, and natimortality) and *Leptospira* spp. infection in sheep ($p = 0.122$).

DISCUSSION

The sero prevalence observed was similar to that reported by Salaberry et al. (2011) in Minas Gerais and Marinho et al. (2012) in São Paulo; however, in comparison to the previous records in the State of Pernambuco, it was found that the results of this study are lower than those described by Mota et al. (1999) and Borba (2004), which reported 57.8% and 33.7% of prevalence, respectively. Mota et al. (1999) used samples collected in Recife, Zona da Mata, and Agreste, not including the semiarid, whereas sampling plans carried out by Borba (2004) covered metropolitan region of Recife, the state Zona da Mata, Agreste, and semiarid of Pernambuco. However, the samples from the Agreste area were only collected in Gravatá, and, from the semiarid, in Floresta. The difference between the results obtained in this study and those registered previously in the State can be related to the geographical regions, sampling plans, and control measures adopted in the properties.

The large number of sites of infection recorded (92.8%) is in agreement with findings from other studies carried out in

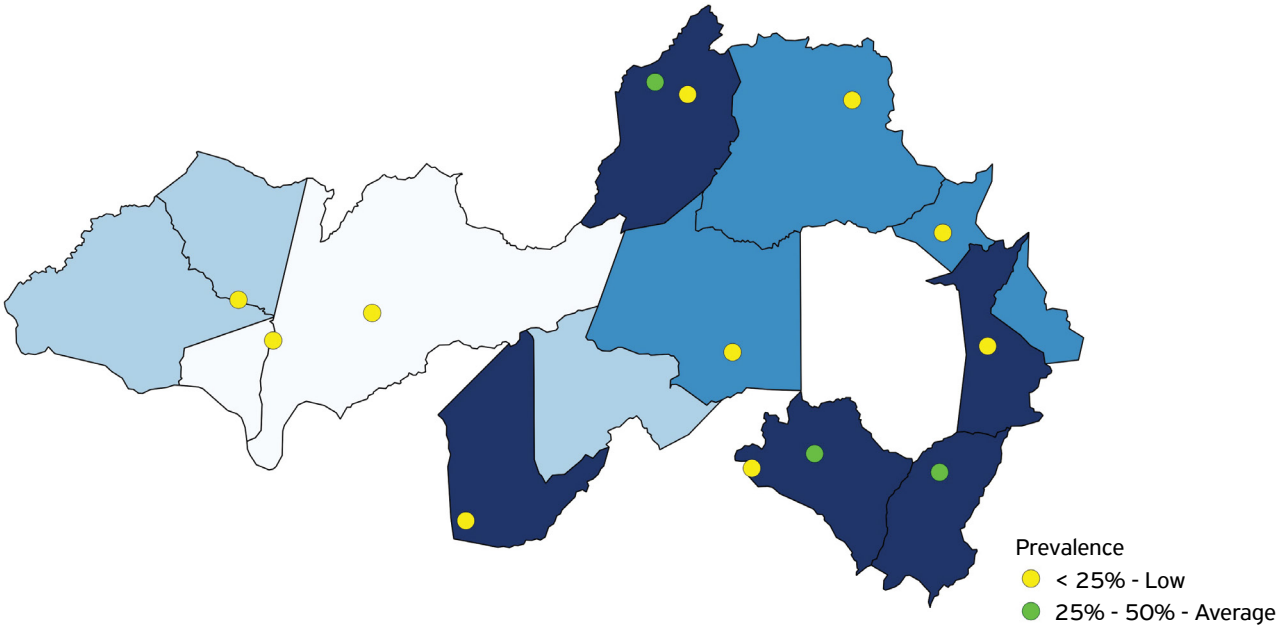

Figure 1. Prevalence of *Leptospira* spp. among sheep in Pernambuco state, 2012.

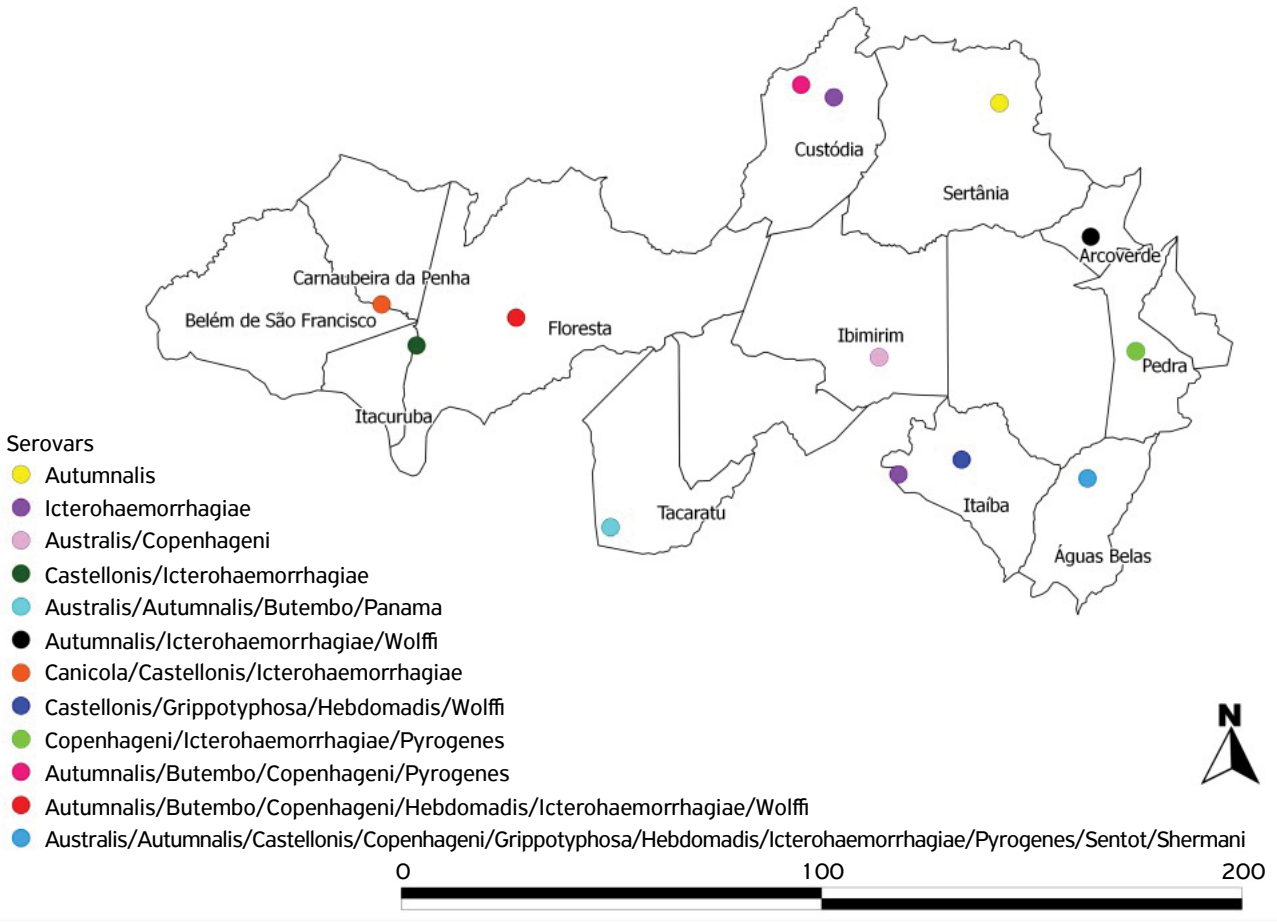

Figure 2. Distribution of *Leptospira* spp. in properties with seropositive sheep in Pernambuco state, 2012.

Table 1. Distribution of *Leptospira* spp. serovars among sheep in Pernambuco state, Brazil.

Serovars	Titer						Total
	1: 100	1: 200	1: 400	1: 800	1: 3.200	1: 6.400	
Australis	1	–	–	–	–	–	1
Autumnalis	18	3	1	1	–	–	23
Butembo	2	2	–	–	–	–	4
Castellonis	4	3	–	–	–	–	7
Copenhageni	2	2	–	–	–	–	4
Grippotyphosa	3	–	–	–	1	–	4
Hebdomadis	3	–	–	–	–	1	4
Icterohamorr-hagiae	9	1	–	–	–	–	10
Panama	–	1	–	–	–	–	1
Pyrogenes	1	2	–	–	–	–	3
Sentot	1	1	–	1	–	–	3
Shermani	–	1	–	–	–	–	1
Wolffi	4	1	1	–	–	–	6
Total	**48**	**17**	**2**	**2**	**1**	**1**	**71**

Table 2. Logistic regression of risk factor associated with *Leptospira* spp. infection among sheep in Pernambuco state, 2012.

Variable	p-Value	OR[a]	95% CI[b]
System (semi-intensive/extensive)	0.029	2.03	1.07 – 3.84
Herd (open/closed)	0.001	2.28	1.39 – 3.72
Access of rodents to grain (no/yes)	0.020	0.55	0.34 – 0.91
Surface water (no/yes)	0.047	0.60	0.37 – 0.99
Gender (male/female)	0.049	0.38	0.14 – 0.99

different regions of the country, as reported by Herrman et al. (2004) in Rio Grande do Sul (83.09%), Aguiar et al. (2010) in Rondônia (80%), Escócio et al. (2010) in São Paulo (100%), Genovez et al. (2011) in São Paulo (100%), Moraes et al. (2012) in Pará (75.0%), and Salaberry et al. (2011) in Minas Gerais.

Variation in the prevalence among properties may be related to factors such as the production system (Herrman et al., 2004), management (Higino et al., 2012), topography, temperature, moisture, precipitation, and contact with other wild animals

or domestic carriers, which contribute to the occurrence rate of infection (Aguiar et al., 2010; Araújo Neto, et al., 2010).

The serovar Autumnalis, found in 32.4% of the animals, has been identified mainly in the Brazilian northeast (Borba, 2004; Higino et al., 2010; Carvalho et al., 2011; Adams et al., 2012). In 2007, *Leptospira nouguchii* species was isolated in apparently healthy sheep, showing that these animals can act as hosts for the serovar Autumnalis (Silva et al., 2007). The serovar Autumnalis was also identified in previous studies as the most frequent carried out in dogs in the city of Patos, PB (Batista et al., 2005) and Monte Negro, RO (Aguiar et al., 2007), coinciding with the results found in sheep in the same locations by Higino et al. (2010) and Aguiar et al. (2010), respectively. It is possible that interspecies relationship has contributed to the agent's maintenance and the transmission between species, showing that studies are needed to identify which species acts as maintenance hosts.

The next most prevalent serovar Icterohaemorrhagiae (14.0%), and also the most widespread in the studied area, is mainly related to the presence of rodents, which reinforces the need for the implementation of programs to control and eliminate rats on properties (Araújo Neto et al., 2010).

Semi-intensive was identified as the system with the higher prevalence among the production systems (21.9%), showing a risk of infection (OR = 2.03). The same result was reported by Mota et al. (1999) in Bezerros, PE. It is possible that the high population density in the semi-intensive system contributes to agent spread through livestock production areas, resulting in a greater challenge for infection control. Promoting routine sanitation and hygiene practices in the properties can contribute in the reduction of the prevalence of leptospirosis in livestock production.

This study identified that in herds where rodents did not have access to grain storage, the risk of infection was lower (OR = 0.55). Therefore, indoors and suspended grain storage are strongly recommended, to avoid access of rodents (Brazil, 2002; FIOCRUZ, 2004), and, consequently, cases of leptospirosis.

The variable gender was also significant for males (OR = 0.38), considered as a protective factor. It was observed that positive males (5 of 54 properties) belonged to four different herds, 100% of which had open and semi-intensive systems. Although the gender variable has been identified as a protective factor, detailed studies are necessary to verify gender-related predisposition, considering that *Leptospira* spp. infection in sheep is closely associated to sanitation and hygienic management.

The present study also found that animals in closed sheep flock have higher chances of infection (OR = 2.28). Closed herds, in which there were no new animal arrivals from other properties, were raised in a semi-intensive system (72.5%), which may be related to the findings in this study, as the system was also considered a risk factor. Because of the possibility of infection by vaginal or semen secretion (Lilenbaum et al., 2008) and placental transmission (Guimarães et al., 1982; 19 of 83 properties), using ram and ewes without proper knowledge of their health status and without preventive antibiotic therapy may have contributed to the infection of the offspring and the increase in frequency for this variable.

In this study, herds in which animals do not had access to stagnant water showed a lower risk of infection (OR = 0.60). According to Andre-Fontain; Ganiere (1990), water is the most important epidemiological factor for leptospirosis. Surface waters can remain contaminated for several weeks under ideal conditions (Brod; Fehlberg, 1992).

In general, it was observed that some of the risk factors identified were related to the production system and the environment's sanitation and hygienic management. Due to the epidemiological complexity of leptospirosis, interventionist programs should be implemented with the purpose of interfere in the life cycle of the bacteria, and its components (Brod; Fehlberg, 1992).

CONCLUSION

The occurrence of leptospirosis in sheep flocks can be influenced by multiple factors. It is important to highlight that this is the first spatial characterization of *Leptospira* spp. infection among sheep in Pernambuco state, and these data provide alternative plans for prevention and control of leptospirosis infection in order to reduce significantly economic losses.

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