

# Risk factors associated with leptospirosis in swine in state of Pernambuco, Brazil

## *Fatores de risco associados à leptospirose em suínos no estado de Pernambuco, Brasil*

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**ABSTRACT:** Leptospirosis is a major public health threat, and swine are one of the most important reservoirs and sources of *Leptospira* infection for man. The aim of this study was to investigate the epidemiological situation of leptospirosis in swine in the state of Pernambuco, Brazil. Blood samples from 265 swine were collected and tested by the microscopic agglutination test (MAT), and an epidemiological form containing questions about animal production characteristics, reproductive management, and sanitary and hygiene aspects of the herd was applied to evaluate infection risk factors. The data were analyzed by means of a logistic regression model. An occurrence of 53.1% (143/265) of swine positives to *Leptospira* spp. was observed. The most common serovars were Icterohaemorrhagiae (39.1%), Pomona (25.9%), and Shermani (14.0%). Factors associated with the infection were stagnant water source ( $p = 0.034$ , *odds ratio* — OR = 2.29; confidence interval of 95% — 95%CI 1.06–4.93), farms where the healthy animals are bred with sick ones (OR = 1.69; 95%CI 1.04–2.75), and the properties with flooded areas (OR = 1.65; 95%CI 1.01–2.68). The risk factors found in this study played an important role in the agent dissemination and should be avoided in a way to control the disease in the herds studied.

**KEYWORDS:** diagnosis; epidemiology; *Leptospira* spp.; pigs; zoonosis.

**RESUMO:** A leptospirose é um grande problema de saúde pública, e os suínos são um dos mais importantes reservatórios e fonte de infecção por *Leptospira* para o homem. O objetivo deste estudo foi investigar a situação epidemiológica da leptospirose em suínos no estado de Pernambuco, Brasil. Amostras de sangue de 265 suínos foram coletadas e testadas pelo teste de microaglutinação (MAT), e foi aplicado um questionário epidemiológico contendo perguntas sobre características da produção, manejo reprodutivo e aspectos sanitários e de higiene do rebanho para avaliar fatores de risco de infecção. Os dados foram analisados por meio de um modelo de regressão logística. Foi observada ocorrência de 53,1% (143/265) de suínos positivos para *Leptospira* spp. Os sorovares mais comuns foram Icterohaemorrhagiae (39,1%), Pomona (25,9%) e Shermani (14,0%). Os fatores associados à infecção foram fonte de água parada ( $p = 0,034$ ; *odds ratio* — OR = 2,29; intervalo de confiança de 95% — IC95% 1,06–4,93), fazendas onde animais saudáveis são criados com os doentes (OR = 1,69; IC95% 1,04–2,75) e áreas inundadas (OR = 1,65; IC95% 1,01–2,68). Os fatores de risco encontrados neste estudo desempenharam papel importante na disseminação do agente e devem ser evitados, de forma a controlar a doença nos rebanhos estudados.

**PALAVRAS-CHAVE:** diagnóstico; epidemiologia; *Leptospira* spp.; porcos; zoonose.

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## INTRODUCTION

Worldwide, leptospirosis is a major public health threat caused by pathogenic spirochetes of the genus *Leptospira* (BHARTI et al., 2003; LEVETT, 2001). *Leptospira* spp. is divided into more than 200 serovars, which are related to particular hosts (LEFEBVRE, 2004). One of the most important reservoirs and sources of infection for man is swine. Besides, leptospirosis is also the major pig-associated zoonosis worldwide (NIWETPATHOMWAT et al., 2006; WASIŃSKI; PEJSKAK, 2010). Beyond all the public health concern, leptospirosis is a disease of economic significance in swine all over the world, because of reproductive losses, such as abortions, stillbirths and weak piglets' birth (BOQVIST et al., 2002; ARENT; ELLIS, 2019).

The disease has been related to both wild and domestic pigs in many countries (KAZAMI et al., 2002; EBANI et al., 2003; GUERRA, 2009). In Brazil, studies were performed to determine the prevalence of leptospirosis and its risk factors in many states, such as Alagoas (VALENÇA et al., 2013), São Paulo (AZEVEDO et al., 2006), Rondônia (AGUIAR et al., 2006), and Paraná (DELBEM et al., 2004). The risk of its transmission from pigs to man is additionally increased by the absence of clinical symptoms and the lack of specific symptoms (WASIŃSKI; PEJSKAK, 2010).

Despite the fact that Brazil has a National Program for Swine Health (Programa Nacional de Sanidade Suídea — PNSS), supervised by a public agency, its implementation is not mandatory for all properties (BRAZIL, 2002; BRAZIL, 2004).

Annually, half million of human leptospirosis cases are estimated to occur worldwide, with the higher incidence in tropical and subtropical areas (WHO, 1999; LEVETT, 2001; BHARTI et al., 2003). In Brazil, leptospirosis is also a major public health problem, with over 9,000 human cases of severe leptospirosis every year (BRAZIL, 2014a).

Studies to improve the knowledge of the risk factors in swine and to better understand its role in the chain of transmission of human leptospirosis are few and especially in the Brazilian northeast. For example, there are none previous studies of leptospirosis in swine in the state of Pernambuco. The purpose of the present study was to investigate the factors that could be associated with *Leptospira* spp. infection in swine from the state of Pernambuco, Brazil.

## MATERIAL AND METHODS

### Sampling

The sample size was calculated as described by SZKLO; NIETO (2012) using the following parameters: swine population of 421,144 animals in state of Pernambuco, Brazil (IBGE, 2010), confidence interval of 95% (95%CI), 5% sampling

error and prevalence of 16%, as described by VALENÇA et al. (2013). The minimum sample size, according to the calculation, would be 207 swine.

From February 2014 to January 2015, we collected 265 blood samples from 16 properties, chosen by convenience, and distributed in eight counties: Alagoinha, Belo Jardim, Capoeiras, Garanhuns, São João, Pedra, Venturosa, and São Bento do Una, all in the state of Pernambuco, Brazil (Fig. 1).

The farmers agreed to the sample taking from the swine by a written informed consent. The blood samples (n = 265) were collected from the jugular vein, stored, properly identified and sent to the laboratory center. None of the properties vaccinated their animals against leptospirosis, because in this region it is not the practice, and also this vaccination is not mandatory (BRAZIL, 2002; BRAZIL, 2004).

An epidemiological form comprising multiple-choice questions about animal production characteristics, reproductive management, and sanitary and hygiene aspects of the herd was applied in each farm. The epidemiological survey contained 13 possible risk factors for *Leptospira* spp. infection, as follows: rearing system (intensive, semi-intensive), water source (stagnant/running/both), breeding with other animal species (yes/no), quarantine (yes/no), empty sanitary (yes/no), healthy animals bred with sick animals (yes/no), flooded areas (yes/no), reproductive management (natural/artificial insemination), origin of animals (farm's own herd/other farms), presence of other domestic animals (yes/no), presence of wild animals (yes/no), presence of rodents (yes/no), and rodent control performed (yes/no).

### Serology test

The sera were tested to diagnose using the microscopic agglutination test (MAT). The antigens used were the following reference strains of 24 serovars of *Leptospira* spp.: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcomb, Cinoptery, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi, Andamana, Patoc, and Sentot. The samples that exhibited decrease of free leptospirosis in the range of 50–100% to the control were exposed to the titration test over a series of geometrical dilutions at the rate of two (TURNER, 1970; COLE et al., 1973). A titer of  $\geq 1:100$  was considered positive (TURNER, 1970). The highest serum dilution capable of agglutinating 50% or more of *Leptospira* than the control was considered the endpoint of the reaction (TURNER, 1970; COLE et al., 1973).

### Statistical analysis

A descriptive analysis was used for calculations of the absolute and relative frequencies related to the serologic tests. A univariate analysis was performed, using Pearson's  $\chi^2$  test or Fisher's

exact test, when necessary, to evaluate the possible risk factors associated with *Leptospira* infection. A logistic regression analysis was carried out, using the results of the serological test as a dependent variable. The explanatory variables were those ones that showed statistical significance  $< 0.20$  in the univariate analysis (HOSMER et al., 2013). This probability was set in order that possible risk factors of the event weren't eliminated from the analysis (HOSMER et al., 2013). The version 23 of the Statistical Package for the Social Sciences for Windows (IBM Corp., 2015) was used to perform the statistical analyses.

## Ethical considerations

The Ethics Committee on Animal Use (Comitê de Ética na Utilização de Animais — CEUA) of the Universidade Federal Rural de Pernambuco (UFRPE) provided scientific and ethical clearance for the present study (license number 044/2013).

## RESULTS

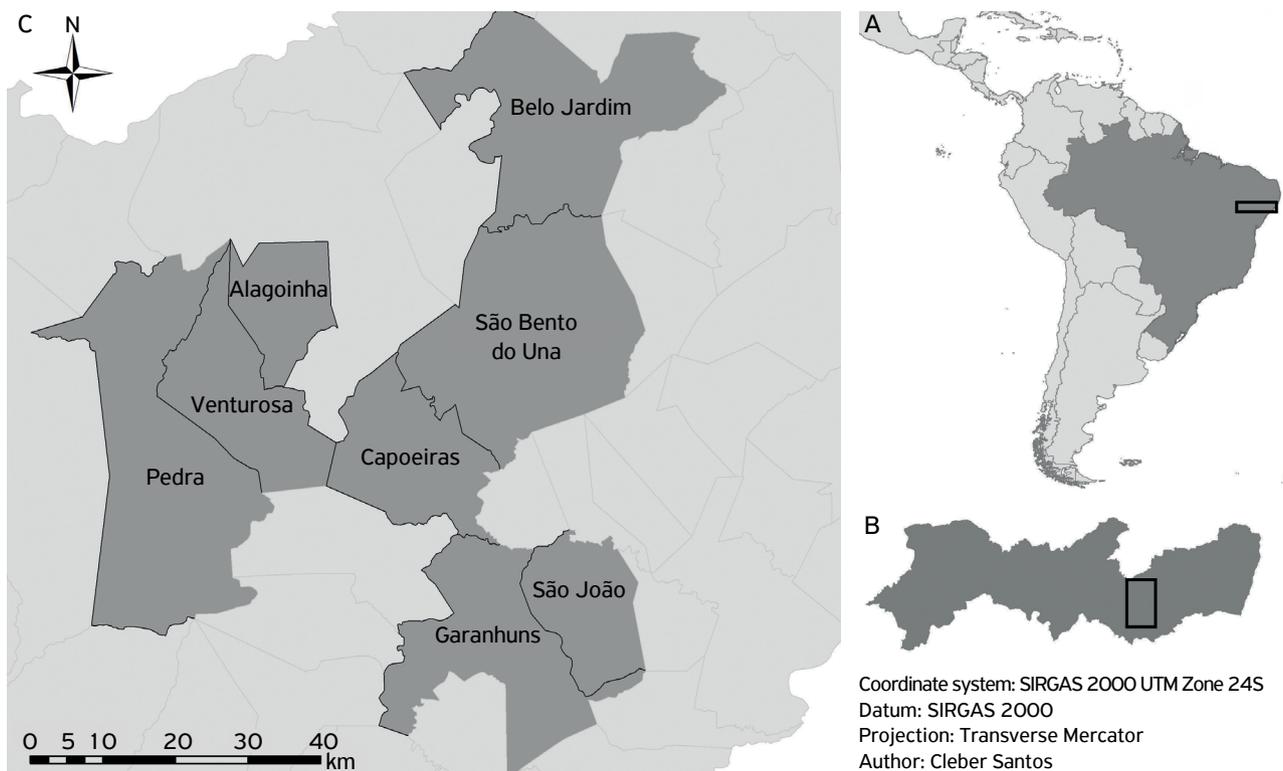
An overall occurrence of 53.1% (95%CI 47.2–59.0) for *Leptospira* spp. infection on swine was observed, with titers varying from 1:100 to 1:1,600 to one or more serovars (Table 1). Considering the number of properties, 93.75% (15 of 16) of the farms had at least one reacting pig. The occurrence on the positive farms ranged from 6.2 to 93.7%.

Most of the titles (34.9%) were 1:100. Among the positive samples, 113 (79.0%) reacted just to one serovar, whereas 30 (21.0%) reacted to more than one serovar. Among the positive samples, the highest prevalence was found for the serovars: Icterohaemorrhagiae (39.1%), Pomona (25.9%), and Shermani (14.0%). The other serovars prevalence varied from 0.0 to 7.7% (Table 1). The most prevalent serovar related to the numbers of outbreaks was Icterohaemorrhagiae, which was present in 93.7% of the properties (15/16), followed by Shermani and Pomona (Table 2). The serovars Canicola, Grippityphosa, Icterohaemorrhagiae, and Tarassovi presented the highest titer (1:1600).

The results of the univariate analysis of the risk factors are presented in Table 3. Logistic regression showed that water source was a risk factor since the risk of infection was 2.29 higher in farms which use stagnant water supply (*odds ratio* — OR = 2.29; 95%CI 1.06–4.93), as the farms where the healthy animals are bred with seek ones (OR = 1.69; 95%CI 1.04–2.75) and the properties with flooded areas (OR = 1.65; 95%CI 1.01–2.68) (Table 3).

## DISCUSSION

This is the first seroepidemiological study to analyze risk factors to *Leptospira* spp. infection in swine in state of Pernambuco. Swine are one of the most important sources of *Leptospira*



**Figure 1.** Study area: (A) Northeast Brazil; (B) Pernambuco state; (C) distribution of municipalities in Pernambuco state.

spp. infection for man and other animals, and since swine in most times do not present clinical symptoms, it is important to discover the occurrence and predominance of *Leptospira* spp. and their serovars in herds, as well to consider the difference among regions (FAINE et al., 1999; ARENT; ELLIS, 2019). The high seroprevalence is dangerous for both swine herd and man, due that an infected animal can excrete large amounts of leptospire in their urine for long periods (up to one year) (BHARTI et al., 2003).

In this mesoregion, 53.1% of the samples were positive in the MAT. Researchers in different countries described prevalence ranging from 0.9 to 66.7% (VAN TIL; DOHOO, 1991; OSAVA et al., 2010; RAMOS et al., 2006; NAITO et al., 2007; WASIŃSKI; PEJSK, 2010). In Brazil, similar results were described in Pernambuco (45.0%) (FAVERO et al., 2002), Rio de Janeiro (66.0%) (RAMOS et al., 2006), and Minas Gerais (47.1%) (OSAVA et al., 2010). LARSSON et al. (1984) found lower positivity in states such São Paulo (12.0%), Santa Catarina (10.1%), and Paraná (8.8%). This kind of results confirms that leptospirosis in swine occurs frequently, and their prevalence fluctuates depending on the region and risk factors. Such variation in seroprevalence may be due to characteristics of the study site, sampling techniques, analysis methods, environmental conditions, and cut-off used in the interpretation of the results.

The high number of positive properties (93.7%) suggests that leptospirosis may have widely disseminated in state of Pernambuco. Nevertheless, further studies are necessary to better understand their occurrence, classify it as an enzootic organism, and determine the real impact of infection in the herd and their impact on public health.

Thirty pigs reacted to more than one serovar. It occurs due to cross-reactions or a true multiple infection (MÉRIEN;

ARTHARID, 2005). Serological tests are the most broadly used to diagnose *Leptospira* spp. (FAINE et al., 1999). The MAT is the standard method for leptospirosis diagnosis, due to his low-cost, and sensibility, but cross-reacting antibodies and vaccination can interfere in the results, especially in low titers, that indicate the past or a chronic infection (FAINE et al., 1999). Therefore, mistakes in the classification of serological status may occur, mainly false-negatives, and the interpretation should be careful (MÉRIEN; ARTHARID, 2005).

In this study, 14 of 24 serovars present in the group of antigens were observed in the swine examined. The higher seroprevalence in the present study was for the serovar Icterohaemorrhagiae. LANGONI et al. (2004) proposed that this positivity occurs in consequence to exposure to rodents,

**Table 2.** Prevalence of *Leptospira* spp. serovars per properties in state of Pernambuco.

Serovar	Proportion of positive farms	Prevalence (%)
Autumnalis	3 of 16	18.7
Bataviae	1 of 16	6.2
Bratislavia	6 of 16	37.5
Canicola	1 of 16	6.2
Castellonis	1 of 16	6.2
Grippotyphosa	3 of 16	18.7
Icterohaemorrhagiae	15 of 16	93.7
Pomona	12 of 16	75.0
Shermani	13 of 16	81.2
Tarassovi	7 of 16	43.7
Wolffi	1 of 16	6.2

**Table 1.** Distribution of the serovars of *Leptospira* spp. in swine from state of Pernambuco.

Serovar	Incidence of titration found										Total	
	100		200		400		800		1600			
	AF	RF	AF	RF	AF	RF	AF	RF	AF	RF	AF	RF
Autumnalis	-	-	2	1.4	2	1.4	-	-	-	-	4	2.8
Bataviae	-	-	1	0.7	1	0.7	1	0.7	-	-	3	2.1
Bratislavia	3	2.1	3	2.1	-	-	-	-	-	-	6	4.2
Canicola	-	-	-	-	-	-	-	-	1	0.7	1	0.7
Castellonis	1	0.7	-	-	-	-	-	-	-	-	1	0.7
Grippotyphosa	-	-	-	-	-	-	2	1.4	1	0.7	3	2.1
Icterohaemorrhagiae	35	24.5	19	13.2	1	0.7	-	-	1	0.7	56	39.1
Pomona	3	2.1	12	8.4	11	7.7	11	7.7	-	-	37	25.9
Shermani	7	4.9	6	4.2	6	4.2	1	0.7	-	-	20	14.0
Tarassovi	1	0.7	3	2.1	5	3.5	1	0.7	1	0.7	11	7.7
Wolffi	-	-	1	0.7	-	-	-	-	-	-	1	0.7
Total	50	34.9	47	32.9	26	18.2	16	11.2	4	2.8	143	100.0

AF: absolute frequency (n); RF: relative frequency (%).

corroborating the study of CHIARELI et al. (2008), because the rodents are the natural hosts of this serovar, and attention should be paid to these numbers, due to this serovar is one of the most associated with severe cases of leptospirosis in

humans (BRAZIL, 2014b). Similar results have been found in different parts of the world, as the most prevalent in those countries (VAN TIL; DOHOO, 1991; KAZAMI et al., 2002; BUCHHOLZ et al., 2016). The serovar Castellonis,

**Table 3.** Analysis of risk factors associated with seroprevalence of *Leptospira* spp. in swine from the state of Pernambuco, Brazil, 2014.

Variable	n	Result	P*	Logistic regression OR (95%CI)	p**
		Positive			
Rearing system					
Intensive	228	118 (51.7%)	.963		
Semi-intensive	37	19 (51.3%)			
Water source					
Running	36	14 (38.9%)		-	
Stagnant	113	67 (59.3%)	.063	2.29 (1.06–4.93)	.034***
Both	116	56 (48.3%)		1.46 (0.68–3.14)	.325
Breeding with other animal species <sup>1</sup>					
Yes	225	119 (52.9%)	.358		
No	40	18 (45.0%)			
Quarantine					
Yes	79	35 (44.3%)	.117		
No	186	102 (54.8%)		1.52 (0.89–2.59)	.117
Healthy animals bred with sick animals					
Yes	140	81 (57.8%)	.034***	1.69 (1.04–2.75)	.034***
No	125	56 (44.8%)			
Flooded areas					
Yes	133	77 (57.9%)	.043***	1.65 (1.01–2.68)	.043***
No	132	60 (45.4%)			
Reproductive management					
Natural breeding	232	120 (51.7%)	.982		
Artificial insemination	33	17 (51.5%)			
Origin of animals <sup>2</sup>					
Farm's own herd	192	94 (48.9%)	.112		
Other farms	61	37 (60.6%)		1.60 (0.89–2.89)	.112
Presence of dogs and cats					
Yes	171	94 (54.9%)	.151	1.44 (0.87–2.40)	.151
No	94	43 (45.7%)			
Presence of wild animals					
Yes	35	17 (48.6%)	.691		
No	230	120 (52.2%)			
Presence of rodents					
Yes	231	121 (52.4%)	.563		
No	34	16 (47.0%)			
Rodent control performed					
Yes	52	22 (42.3%)	.131	1.60 (0.86–2.95)	.132
No	213	115 (54.0%)			

\* $\chi^2$  test; \*\*logistic regression model; \*\*\*statistically significant at 5%; OR: odds ratio; 95%CI: confidence interval of 95%; <sup>1</sup>e.g., horses and cattle; <sup>2</sup>different base (253).

also detected in the study, has rodents as the most common hosts (AGUIAR et al., 2006).

In this study, the presence of rodents on the farms was observed in most facilities (87.1%), as well as the absence of rodents' control (80.4%). Despite there was no statistical association between control of rodents and *Leptospira* spp. infection, other studies, such DELBEM et al. (2002), found that farms that do not perform rodents control show higher chances (7.8 times) of infection. Serovar Pomona, the second most prevalent in this study, is host-adapted to swine and it is the main reason for economic losses in swine farms. Due to the evident occurrence of this serovar in the present study, it is suggested that its role in herds should not be overlooked (ARENT; ELLIS, 2019; RAMOS et al., 2006; NAITO et al., 2007).

There was a change in the prevalence pattern of the serovars. Pomona, commonly cited as the predominant all over the world, was substituted by the serovar Icterohaemorrhagiae, corroborating the hypothesis that failures in the rodents control are occurring in the properties and their environments (DELBEM et al., 2002; FAVERO et al., 2002; ARENT; ELLIS, 2019). The serovar Shermani was the third most prevalent in this research, and such prevalence was unexpected, since there are few studies describing this serovar in Brazil, what confirms the heterogeneity of serovars (AZEVEDO et al., 2006). However, neither clinical signs nor reproductive losses associated with this serovar have ever been reported in pigs (DELBEM et al., 2004; AZEVEDO et al., 2006).

The logistic regression showed that water sources with stagnant water (OR = 2.29; 95%CI 1.06–4.93) are a risk factor for leptospirosis. Those sources, especially the ones that are non commonly cleaned up, may be used to other animals, mainly the rodents, and, therefore, can be related to their infestation (BOQVIST et al., 2002; MCBRIDE et al., 2005; CHANNON et al., 2006).

Higher prevalence was observed in animals with flooded areas and with access to surface water, which facilitates the

transmission of the agent, and consequently it is a risk factor for leptospirosis (OR = 1.65; 95%CI 1.01–2.68). Most of risk factors are water-related, a basic need for leptospires surviving (COSTA et al., 2015). Many studies cited water as the most important factor to leptospirosis, and the stagnant water as the main transmission source, due its long period of resistance in that conditions (BARCELLOS; SABROZA, 2001; TRUEBA et al., 2004; MCBRIDE et al., 2005). DOUGLIN et al. (1997) found that people who walk in areas with stagnant water has 25.62 more chances to be positive in leptospirosis diagnosis.

The cohabiting of healthy animals with sick animals in the same site was expected to be a risk factor to leptospirosis (OR = 1.69; 95%CI 1.04–2.75), because this may cause direct or indirect transmission through urine, other body fluids and fomites (FAINE et al., 1999; BOQVIST et al., 2002; MCBRIDE et al., 2005; COSTA et al., 2015). Despite there was no statistical association between wild animals, dogs, cats and other species and leptospirosis, many studies found that significant (BOQVIST et al., 2002; DELBEM et al., 2004; SOTO et al., 2007).

Further studies with the use of polymerase chain reaction (PCR) and culture are necessary to better understand the *Leptospira* spp. patterns, such as to determine the animals that are eliminating the bacteria and may play a role in the transmission from them to those ones that have only been exposed to the agent. As SOTO et al. (2007) emphasize in their studies, it is fundamental to determine the role of swine in the transmission chain of leptospirosis to humans.

It is suggested that the farms that maintain the healthy animals bred with sick animals change this type of management, as the ones with access to flooded areas in a way to decrease the risk of infection. It is important to pay special attention to the water sources, since the stagnant water was identified as a risk factor to leptospirosis, and we strongly recommend to the farmers who have the water source with stagnant water to change it.

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