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Epidemiological survey of leptospirosis in collared peccaries (*Pecari tajacu* Linnaeus, 1758) in legal breeding sites in some states in the Northeast region, Brazil

Levantamento epidemiológico da leptospirose em catetos (*Pecari tajacu* Linnaeus, 1758) de criadouros legalizados em alguns estados da região Nordeste do Brasil

Estudio epidemiológico de leptospirosis en pecarí de colar (*Pecari tajacu* Linnaeus, 1758) de criaderos legales en algunos estados de la región Nordeste de Brasil

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Abstract
The collared peccary (*Pecari tajacu* Linnaeus, 1758) is considered an animal of economic viability for trading and potentially productive for meat, being important the knowledge about the health of this species. Thus, the objective of the research was to carry out a cross-sectional study of leptospirosis in captive collared peccary (*Pecari tajacu*) from the states of Paraíba, Rio Grande do Norte and Piauí, northeast region of Brazil, using serological and molecular techniques. Serum samples from 48 animals were tested using the microscopic agglutination test (MAT) technique. In the samples of vaginal and preputial fluid, the Polymerase Chain Reaction (PCR) was performed. Four animals (8.3%) were seroreactive for *Leptospira* sp. with reaction to serogroup Icterohaemorraghiae and negative in PCR. There was association between the occurrence of leptospirosis and the intensive breeding system (*odds ratio*=63.00; 95%CI=4.3-910.6; P=0.002). The seroreactivity for leptospirosis suggests that, at some point, these animals were infected by sources of infection within the farm itself. It was also possible to observe the importance of knowing the serogroups prevalent in this species in the studied region, which allows the establishment of adequate strategies for its control, thus prioritizing the balance in the human-animal-environment relationship.
Keywords: Tayassuidae; *Leptospira* spp.; PCR; Serology; Zoonosis.

Resumo
O cateto *(Pecari tajacu* Linnaeus, 1758) é considerado um animal de viabilidade econômica para comércio e com potencial produtivo para carne, sendo importante o conhecimento sobre a sanidade. Assim, o objetivo dessa pesquisa foi realizar um estudo transversal da leptospirose em catetos (*Pecari tajacu*) de cativeiro oriundos dos estados da Paraíba, Rio Grande do Norte e Piauí, região nordeste do Brasil através de técnicas sorológicas e moleculares. Amostras de soro de 48 animais foram testadas pela técnica de soroaglutinação microscópica (MAT). Nas amostras de fluido vaginal e prepucial foi realizada pela reação da cadeia de polimerase (PCR). Quatro animais (8,3%) foram sororeativos para *Leptospira* sp. com reação para o sorogrupo Icterohaemorraghiae e negativos na PCR. Foi verificada associação entre a ocorrência de leptospirose e o sistema de criação intensivo (*odds ratio*=63,00; 95%IC=4,3-910,6; P=0,002). A sororeatividade para leptospirose sugere que em algum momento esses animais foram infectados através de fontes de infecção dentro do próprio criatório, sendo indicada a detecção dessas fontes de infecção aliada a medidas corretivas. Também foi possível observar a importância de se conhecer os sorogrupos prevalentes nesta espécie na região semiárida do Brasil, o que permite o estabelecimento de estratégias adequadas para o controle da mesma, priorizando assim o equilíbrio na relação humano-animal-ambiente.
Palavras-chave: Tayassuidae; *Leptospira* spp.; PCR; Sorologia; Zoonose.

Resumen
El pecarí de collar (*Pecari tajacu* Linnaeus, 1758) es considerado un animal de viabilidad económica para el comercio y con potencial productivo para la carne, siendo importante el conocimiento sobre salud. Así, el objetivo de esta investigación fue realizar un estudio transversal de leptospirosis en pecarí de collar (*Pecari tajacu*) en cautiverio de los estados de Paraíba, Rio Grande do Norte y Piauí, región nordeste de Brasil, usando técnicas serológicas y moleculares. Se analizaron muestras de suero de 48 animales usando la técnica de aglutinación microscópica de suero (MAT). En las muestras de líquido vaginal y prepucial se realizó mediante la reacción en cadena de la polimerasa (PCR). Cuatro animales (8,3%) fueron seroreactivos para *Leptospira* sp. con reacción al serogrupo Icterohaemorraghiae y negativo en PCR. Hubo una asociación entre la aparición de leptospirosis y el sistema de cría intensiva (razón de posibilidades=63,00; 95%IC=4,3-910,6; P=0,002). La serorreactividad para la leptospirosis sugiere que en algún momento estos animales se infectaron a través de fuentes de infección dentro de la instalación de cría, y está indicada la detección de estas fuentes de infección combinada con medidas correctivas. También fue posible observar la importancia de conocer los serogrupos prevalentes en esta especie en la región semiárida de Brasil, lo que permite establecer estrategias adecuadas para su control, priorizando así el equilibrio en la relación humano-animal-ambiente.
Palabras clave: Tayassuidae; *Leptospira* spp.; PCR; Serología; Zoonosis.

1. Introduction

The collared peccary (*Pecari tajacu* Linnaeus, 1758) is a medium-sized mammal belonging to the order Artiodactyla, suborder Suiformes and family Tayassuidae (Furtado, 2014). It is found from the southern United States, throughout Central America and in much of South America, especially in Brazil, where it is widespread (Margarido & Mangini, 2001; Sonner et al., 2004). It occupies several types of environments, such as tropical or temperate forests, deserts, and swamps, which

highlights the euritopic character of this species, what makes these animals have no need for major environmental changes in order to implement a breeding site (Bodmer & Sowls, 1993).

Pecari tajacu stands out, in Brazil, as an animal of economic viability for trade and with great productive potential for meat, being the sixth most consumed exotic meat in the country (Ribeiro, Carvalho, Peruquetti, Medeiros & Freitas, 2016), with its commercial creation being legal according to the Normative Instruction (Annex 169/2008) from the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis [IBAMA] (2008).

Collared peccaries may be susceptible to the same infections that affect domestic swine and may act as reservoirs for some diseases such as leptospirosis, which may have a significant impact on the production and reproduction of the species (Furtado, 2014). Furthermore, the observation and study of animal pathologies enable the understanding of possible interactions with humans, which is of great importance in public health (Albuquerque et al., 2016).

According to Coleman (2000), Sales et al. (2012) and Centers for Disease Control and Prevention [CDC] (2019), leptospirosis is an acute to chronic zoonosis that affects several species of domestic animals, humans, and wild animals, such as artiodactyls. It is caused by an aerobic, mobile, spirited *Leptospira*, demanding in terms of microbiological culture (Genovez, 2016). However, data on infection by *Leptospira* spp. in the collared peccary are limited, mainly in the Northeast region of Brazil (Minervino et al., 2018).

Thus, the study of the epidemiology of leptospirosis in the species *Pecari tajacu* is important for understanding its role as a natural reservoir, characterizing the circulation of this infectious agent between wild and domestic species, supporting the development of actions that will minimize the negative impact of this for public health. Therefore, the objective of this work was to carry out a cross-sectional study of leptospirosis in collared peccaries (*Pecari tajacu*) in captivity in the States of Paraíba, Rio Grande do Norte and Piauí, Northeastern region of Brazil, using serological and molecular techniques.

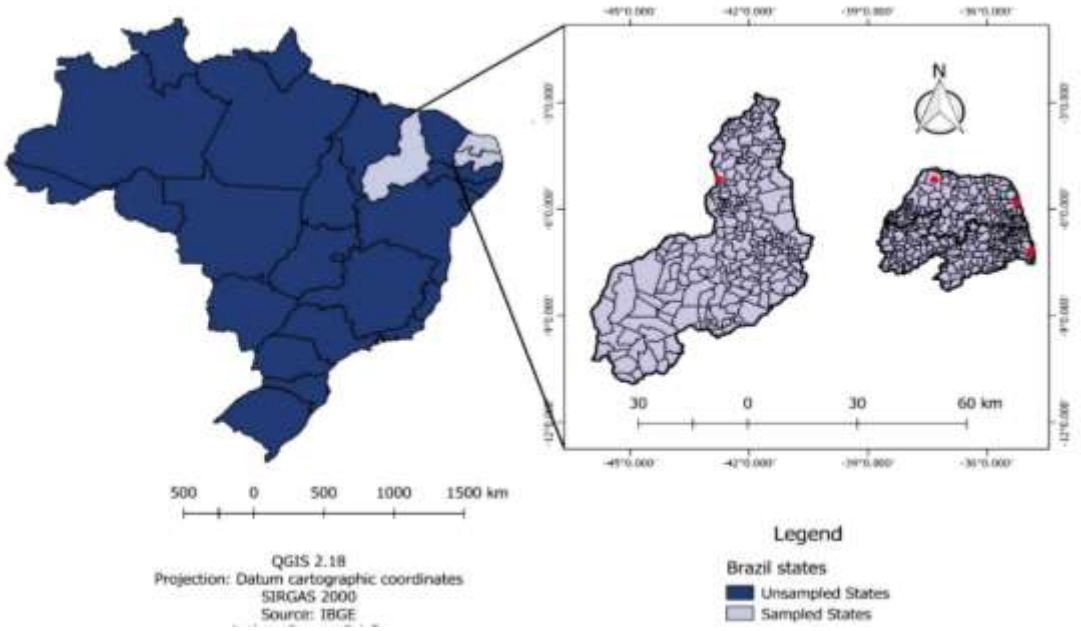
2. Methodology

2.1 Sampling and field activities

The research was carried out with a qualitative and quantitative described by Pereira et al. (2018). The methodological protocols for this study were approved by the Sistema de Autorização e Informação em Biodiversidade (SISBio under n° 36263-5) and the Comitê de Ética em Pesquisa no Uso de Animais (CEUA) of the Centro de Saúde e Tecnologia Rural (CSTR) from the Universidade Federal de Campina Grande (UFCG), Campus in the city of Patos, Paraíba, Brazil (n° 073-2017 and 005-2018).

48 collared peccaries were used, 18 females and 30 males, kept in captivity in four distinct breeding sites (A - D) located in three states of Northeast region of Brazil, identified with their respective geographical coordinates, which are: a zoo with five Collared peccaries(Location A: -71142685S, - 348774146O) in the municipality of João Pessoa, Paraíba; a breeding site with five Collared peccaries (Location B: -5731769S, -35204213O) in the city of Natal, Rio Grande do Norte; a scientific breeding site with nineteen Collared peccaries (Location C: -5213470S, -37310019O) in Mossoró, Rio Grande do Norte and a scientific and commercial breeding site also with nineteen Collared peccaries (Location D: -50478053S, -427779826O) in Teresina, Piauí (Figure 1). For the selection of sampled breeding sites, a previous survey was carried out with legal institutions and 100% of the identified breeding sites entered the study.

Figure 1 - Collection sites for blood, vaginal and foreskin fluid from collared peccaries (*Pecari tajacu*) for diagnosis of brucellosis and leptospirosis.



Fonte: Laboratório de Doenças Transmissíveis, UFCG (2019).

After physical containment of the animal with the handgrip and/or chemical with midazolam 0.3 to 0.5 mg / kg, IM (Furtado, 2014), blood samples were collected by puncture of the lateral saphenous vein and transported in isothermal boxes to the Laboratório de Doenças Transmissíveis at the Centro de Saúde e Tecnologia Rural of the Universidade Federal de Campina Grande (LDT / CSTR / UFCG), in Patos, tate of Paraíba, where they were drained and stored at -20° C until serological tests were performed. Collections of vaginal and preputial fluid were also made with the aid of sterile swabs, which were stored in microtubes containing 1.5 ml of 0.9 % sodium chloride solution and sent to the Laboratório de Biologia Molecular do Semiárido (BIOMOL/CSTR/UFCG), in Patos, Paraíba, for DNA extraction and subsequent Polymerase Chain Reaction (PCR). An epidemiological questionnaire was applied to those responsible for the breeding of collared peccaries, which was designed to offer variables that sought to verify the absence or presence of some practices and conditions that may act as a parameter indicating the chances of animals being seroreactive for the disease.

2.2 Serological diagnosis of *Leptospira* spp.

The serological diagnosis of leptospirosis was performed using the microscopic agglutination test (MAT) technique, a test recommended by the World Organization for Animal Health [OIE] (2019), with a collection of 24 live antigens, represented by the serogroups Castellonis, Javanica, Tarassovi, Whitcombi, Australis, Autumnalis, Bataviae, Bratislava, Canicola, Copenhageni, Grippotyphosa, Hardjo (strains Hardjoprajitno and Hardjobovis), Hebdomadis, Pomona, Icterohaemor, Sentot, Wolffi, Pyrogenes, Butembo, Cynopteri, Panama, Shermani, Andamana, and Patoc. The sera were screened at the dilution of 1:50 (Cordeiro, Oliveira & Vieira, 2017) and those with 50 % or more agglutination were titrated by examining a series of geometric dilutions of ratio two. The serum titer was the reciprocal of the highest dilution that showed a positive result. Antigens were examined under a dark field microscope, prior to testing, to verify mobility and the presence of autoagglutination or possible contaminants.

For differential diagnosis, the Rose Bengal plate test (RBT) test recommended by for the Programa Nacional de Controle e Erradicação da Brucelose e da Tuberculose Animal was used [PNCEBT] (2017).

2.3 Molecular diagnosis of *Leptospira* spp.

The DNA contained in the vaginal and foreskin swabs was extracted using the Dneasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the recommendations from the manufacturer. The polymerase chain reaction (PCR) was performed with the primers LipL32-45F (5′-AAG CAT TAC CGC TGG TG-3 ′) and LipL32-286R (5′-GAA CTC CCA TTT CAG CGA TT-3 ′), to amplify the LipL32 gene, which is specific for pathogenic *Leptospira* spp.. As a positive control, Pomona serovar Kennewicki sample was used and ultrapure water was used as a negative control (Stoddard, Gee, Wilkins, McCaustland & Hoffmaster, 2009).

2.4 Statistical analysis

The analysis of factors associated with seroreactivity for *Leptospira* spp. was performed with data collected from epidemiological questionnaires applied to breeders and was carried out in two stages: univariate and multivariate analysis.

In the univariable analysis, two groups of animals were formed - reactive and non-reactive - which were compared against the analyzed variables: sex (male, female); age (3-12 months, 13-24 months, 25-48 months, > 48 months); general management: Type of destination (preservation, commercial), breeding system (intensive, semi-intensive, extensive), access to water (natural source, drinking fountains), water source (water company, tubular well) contact with other animals (no, yes); sanitary/reproductive management: deworming (no, yes), quarantine (no, yes), separate picket for females in the delivery and/or postpartum phase (no, yes), there is control of rats (no, yes), animal has contact with dams/flooded areas (no, yes), tests for leptospirosis diagnosis (no, yes), natural mating (no, yes); and infrastructure: management center (no, yes), type of enclosure (beaten earth floor, slatted, cemented, other).

Those variables that presented $P \leq 0.2$ by the chi-square test or Fisher's exact test (Zar, 2010) were selected for multivariate analysis, using multiple logistic regression (Hosmer & Lemeshow, 2000). The level of statistical significance was set at 0.05 and calculations were performed using the Statistical Package for the Social Sciences (SPSS) software® version 20.0.

3. Results

From 48 samples submitted to the MAT, four (8.33 %) were positive, being two females and one male from site A and one female from site D for anti-*Leptospira* spp. Agglutinins, showing seroreactivity for the serogroup Icterohaemorraghiae with titers ranging from 1:50 to 1:200 (Table 1).

Table 1 - Result of serology for Leptospirosis in collared peccaries (*Pecari tajacu*) in the states of Paraíba, Rio Grande do Norte and Piauí, Northeastern region of Brazil, between the years 2017 and 2018.

Farm	Nº positive/ Nº tested	Serogroup	Titration
A	3/5	Icterohaemorrhagiae; Icterohaemorrhagiae; Icterohaemorrhagiae	100 200 200
B	0/5	___	___
C	0/19	___	___
D	1/19	Icterohaemorrhagiae	50

Fonte: Laboratório de Doenças Transmissíveis, UFCG (2019).

The results of the analysis of risk factors are presented in Table 2, where the selected variables (P≤ 0.2) for the multiple analysis were: sex, breeding system, contact with other animals, quarantine, separate paddock for females in the delivery phase and/or postpartum, natural mating and type of enclosure. In the multiple analysis, it was verified that intensively bred animals had a higher chance of seroreactivity (*odds ratio=63.00; 95% IC=4.3-910.6; P=0.002*) (Table 3).

Table 2 - Univariate analysis for odds ratio associated with seroprevalence of Leptospirosis in collared peccaries (*Pecari tajacu*) in the states of Paraíba, Rio Grande do Norte and Piauí, Northeastern region of Brazil. [A] Variables selected and used in the multiple analyze (P ≤ 0.2).

Variables	Categories	Nº of Sampled Animals	Nº of Positive Animals (%)	*P*
Sex	Male	30	1 (33)	0.106 [A]
	Female	18	3 (16.7)	
Age	3-12 months	3	0	0.646
	25-48 months	5	0	
	> 48 months	40	4(10)	
Type of destination	Preservation	29	3(10.3)	0.533
	Commercial	19	1(5.3)	
Breeding system	Intensive	5	3 (60)	≤ 0.001 [A]
	Semi-intensive	43	1 (2.3)	
Acess to water	Natural source	29	3(10.3)	0.533
	Drinking fountains	19	1(5.3)	
Water source	Water company	29	3(10.3)	0.533
	Tubular well	19	1(5.3)	
Contact with other animals	No	38	1 (2.6)	0.005 [A]
	Yes	10	3 (30)	
Deworming	No	19	1(5.3)	0.533
	Yes	29	3(10.3)	
Quarantine	No	5	3 (60)	≤ 0.001 [A]
	Yes	43	1 (2.3)	
Separate picket for females in the delivery and/or postpartum phase	No	10	3(30)	0.005 [A]
	Yes	38	1(2.6)	
There is control of rats	No	24	1(4.2)	0.296
	Yes	24	3(12.5)	
Animal has contact with dams/flooded areas	No	29	3(10.3)	0.533
	Yes	19	1(5.3)	
Tests for leptospirosis diagnosis	No	43	4(9.3)	0.476
	Yes	5	0	
Natural mating	No	10	3 (30)	0.005 [A]
	Yes	38	1 (2.6)	
Management center	No	5	0	0.476
	Yes	43	4(9.3)	
Type of enclosure	Beaten Earth floor	10	3 (30)	0.005 [A]
	Slatted	0	0	
	Cemented	38	1 (2.6)	

Fonte: Laboratório de Doenças Transmissíveis, UFCG (2019).

Table 3 - Multivariate analysis for odds ratio associated with seroprevalence of Leptospirosis in collared peccaries (*Pecari tajacu*) in the states of Paraíba, Rio Grande do Norte and Piauí, Northeastern region of Brazil. *R^2 de Nagelkerke= 0.481*.

Variable category	Coefficient estimates	Standard error	Wald Chi-square	Degree of Freedom	*Odds Ratio* (OR)	95% CI	*P*
Type of destination	4.143	1.363	9.243	1	63.00	4.359- 910.6	0.002

Fonte: Laboratório de Doenças Transmissíveis, UFCG (2019).

In the molecular analysis for the LipL32 gene of *Leptospira* spp., through samples of vaginal and preputial fluid, all animals were negative in PCR. In the differential diagnosis for *Brucella abortus*, there were no positive reactions, excluding the need for a confirmatory test of 2-mercaptoethanol (2-ME).

4. Discussion

The diagnosis of leptospirosis has already been described in some wild animals, including collared peccaries (*Pecari tajacu*), from various regions of Brazil and the USA (Luna-Alvarez, Moles-Cervantes, Torres-Barranca & Gual-Sill, 1996; Ito et al., 1998; Jori, Galvez, Mendoza, Cespedes & Mayor, 2009), but this is the first study in the Northeast region of Brazil for this species. The four positive animals reacted to the serogroup Icterohaemorraghiae, which has been identified as the main responsible for the clinical cases of human leptospirosis in Brazil and the rodent *Rattus norvegicus* is considered the most relevant host (Bharti et al., 2003; Souza et al., 2006; Martins & Spink, 2020). There is an important role for rodents in the transmission of serogroup Icterohaemorraghiae to wild animals that live in captivity, since they are present in agglomeration sites, making contact more favorable, according to the Corrêa et al. (2004) and Ministério da Saúde [MS] (2019).

Regarding the seroreactivity of the collared peccary for leptospirosis, a research was carried out in *Pecari tajacu* from Pará, through MAT, in which four (9.8 %) animals were positive for the serogroup Autumnalis (Mayor et al., 2006). In Mexico, two collared peccaries were positive for the Icterohaemorrhagie and Panama serogroups ((Luna-Alvarez, Moles-Cervantes, Torres-Barranca & Gual-Sill, 1996). In Colombia it was reported that 39 (78 %) free-living collared peccaries showed a high prevalence for serogroups Australis, Icterohaemorrhagiae, Grippotyphosa, Canicola, and Pomona (Montenegro et al., 2018). Thus, it is possible to indicate the participation of these animals as links in the epidemiological chain of the disease and, probably, the lower prevalence in this study may be associated with the better sanitary control offered to captive animals (8.33 %), differently from free-living ones.

The highest frequency of positive *Pecari tajacu* was identified at the zoo (site A), in which the breeding system is intensive. However, the high value of the o*dds ratio* does not reflect the real chance that an intensively bred animal will be seroreactive and this was probably due to the small number of samples included in this study. However, as the animals are confined, humidity may favor the maintenance of the bacteria in the breeding paddocks, as well as the accumulation of water near the enclosures. In addition to the contact of rodents with the collared peccaries due to the breeding sites, which are usually located in the urban perimeter with areas of extensive vegetation, facilitating the survival of these synanthropic animals.

In the molecular analysis for *Leptospira* spp. this research differs from another carried out using three captive collared peccaries in the municipality of Ilha Solteira in the state of São Paulo, where one (33.3 %) *Pecari tajacu* was positive for PCR, indicating infection with the pathogen, without developing the disease (Paixão et al., 2011). It should be noted that the excretion of *Leptospira* spp. in the urine is intermittent; thus, negative results in PCR do not exclude the possibility that the

animal is a host. Therefore, periodic tests may be necessary for the proper detection of all carriers in a breeding site (Lilenbaum et al., 2009).

5. Conclusion

The seroreactivity for leptospirosis suggests that at some point these animals were infected through sources within the intensive environment itself, such as contact with synanthropic hosts or pools of water. In these cases, it is recommended to detect these sources in the environment, combined with corrective measures, in addition to a new serological investigation to verify the ineffectiveness or not of the intervention in the environment.

In addition, it was possible to observe the importance of knowing the serogroups prevalent in this species in three states investigated in the Northeast region, which allows the establishment of appropriate strategies for effective control, thus prioritizing the balance in the human-animal-environment interaction.

For future research on leptospirosis in collared peccaries, if possible, it is suggested to schedule collections in the rainy periods as it is more conducive to the transmission of the disease at this time of the year. In addition, in the event of the death of an animal, it should investigate the identification of bacteria in the tissues through immunohistochemistry or other anatomopathological analyzes.

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