DOI: 10.5433/1679-0359.2019v40n6Supl3p3541

Leptospirosis in donkeys (*Equus asinus)* destined for slaughter and export

Leptospirose em asininos (*Equus asinus)* destinados ao abate para exportação

Davidianne de Andrade Morais[1]; Dinamérico de Alencar Santos Júnior[1]; Bruno Cesar Nunes[1]; Diego Figueiredo Costa[1]; Maira Porto Viana[1]; José Dêvede da Silva[1]; Severino Silvano dos Santos Higino[2]; Sérgio Santos Azevedo[2]; Clebert José Alves[2]*

Abstract

Brazil is the eighth largest exporter of equidae meat in the world. Most donkey meat is obtained from discarded animals, raising concerns about their health status, particularly regarding zoonotic infections such as leptospirosis. Thus, this study aimed to determine the frequency of anti-*Leptospira* sp. antibodies in Northeastern donkeys at two properties specializing in producing donkeys for slaughter and export to the Chinese market, in the municipalities of Parnamirim (A) and Araripina (B), both located in the mesoregion of the Sertão of Pernambuco, Northeastern Brazil. The serum of 349 donkeys was collected, including 147 males and 202 females, aged 3 to 12 years. All animals were subjected to the Microscopic Agglutination Test (MAT) using a cut-off point of 1:50. A total of 19.8% seropositive animals were obtained. The Icterohaemorrhagiae serogroup was the most found in this study (40.6%), followed by Australis (27.5%), Autumnalis (8.7%), Sejroe (8.7%), Pomona (8.7%), Celledoni (2.9%), and Tarassovi (2.9%). A higher overall rate of seropositivity was found in donkeys from property B (2.2.4%) in the municipality of Araripina. In the association analysis, it was observed that animals less than 9 years of age were more likely to be seropositive, this may be due to the acquisition of resistance to *Leptospira* sp. as age advances. A significant number of donkeys seropositive for *Leptospira* sp. was found, thus, breeding these animals for slaughter and export requires implementing prophylactic measures.
Key words: Equids. Infection. Microscopic Agglutination Test.

Resumo

O Brasil é o oitavo maior exportador de carne equídea do mundo. A carne de asininos provém, na maioria das vezes, de animais de descarte, havendo preocupações com a sua saúde, principalmente quanto às infecções zoonóticas que podem acometê-los, dentre elas a leptospirose. Dessa forma, objetivou-se com esse estudo determinar a frequência de anticorpos anti-*Leptospira* sp. em asininos da raça Jumento Nordestino em duas propriedades especializadas em envio de asininos para abate e exportação para o mercado chinês, nos municípios de Parnamirim (A) e Araripina (B), ambas localizadas na Mesorregião do Sertão de Pernambuco, Nordeste do Brasil. Foi utilizado o soro de 349 asininos, sendo 147

[1] Discentes, Curso de Doutorado, Programa de Pós-Graduação em Ciência e Saúde Animal, Universidade Federal de Campina Grande, UFCG, Patos, PB, Brasil. E-mail: davidianneandrademorais@gmail.com; juniordinamerico@yahoo.com.br; bruno.cnunes@hotmail.com; diegoveter@hotmail.com; mairaporto.veterinaria@gmail.com; dvd.12@hotmail.com

[2] Profs., Programa de Pós-Graduação em Ciência e Saúde Animal, UFCG, Patos, PB, Brasil. E-mail: higinosss@gmail.com; sergio@vps.fmvz.usp.br; clebertja@uol.com.br

* Author for correspondence

Received: Mar. 24, 2019 - Approved: Aug. 22, 2019

machos e 202 fêmeas com idade entre 3 e 12 anos. Todos os animais foram submetidos a técnica de Soroaglutinação Microscópica (MAT), utilizando-se o ponto de corte 1:50. Foi obtida uma frequência de 19,8% soropositivos. O sorogrupo Icterohaemorrhagiae foi o mais frequente nesse estudo (40,6%), seguido por Australis (27,5%), Autumnalis (8,7%), Sejroe (8,7%), Pomona (8,7%), Celledoni (2,9%) e Tarassovi (2,9%), com frequência de soropositividade maior nos asininos provenientes da propriedade B (22,4%), no município de Araripina. Na análise de associação observou-se que os animais com idade inferior à nove anos tiveram maiores chances de serem soropositivos, podendo ser atribuída à aquisição de resistência aos patógenos com o avançar da idade. Houve uma significativa detecção de asininos soropositivos para *Leptospira* sp., sendo necessária a implantação de medidas profiláticas nas criações desses animais destinados ao abate para exportação.
Palavras-chave: Equídeos. Infecção. Soroaglutinação Microscópica.

Introduction

Equids are part of a heritage of historical importance (SALLES et al., 2013), because they were fundamental in the development of humanity (PEREIRA et al., 2015). Equines include donkeys (*Equus asinus)* (GRINDER et al., 2006), a species that is highly resistant to diseases (CARRIJO JUNIOR; MURAD, 2016). Donkeys make up a vast patrimony of social, cultural, ecological, and economic importance (ULIANA et al., 2016), the latter mainly referring to the production of meat for human consumption (LAUS et al., 2015). The use of donkeys for meat production aided Brazil in becoming the eighth largest exporter of equidae meat in the world (SANTOS et al., 2016).

One of the breeds of donkeys originated in Brazil is the Northeastern Donkey (*Equus asinus*), a highly rustic animal and well adapted to the adverse conditions of the Brazilian semi-arid region (LOURO et al., 2006). Although 90% of the country's donkey population is concentrated in Northeastern Brazil, a gradual reduction of this percentage has been observed over the years (IBGE, 2012). The population of donkeys for meat production in Brazil is primarily composed of discarded animals, with little concern for their health status, particularly regarding infection with zoonotic pathogens, such as leptospirosis (SANTOS et al., 2016).

Classified as a bacterial anthropozoonosis that affects domestic, wild, and synanthropic animals, leptospirosis is caused by pathogenic species of the genus *Leptospira* (BATISTA et al., 2016), with many serogroups identified, each of them having its preferred hosts (SANTOS et al., 2018). Some authors, such as Pinna et al. (2008), consider equids as the main reservoirs for the Australis serogroup. Although these animals are generally asymptomatic (SANTOS et al., 2012), Braga et al. (2011) point out some indicators of infection by *Leptospira* sp. in equids: abortion, weak neonates or premature fetuses, and recurrent uveitis, also known as periodic ophthalmia. This disease is considered an occupational threat to workers who are routinely exposed to open water sources or animals, including veterinarians, ranchers, slaughtermen, and meat inspectors (GREVEMEYER et al., 2017); contact with the pathogen occurs via placental remains, fetal tissues, urine, or water contaminated with these tissues or fluids (PAIXÃO et al., 2016).

Leptospirosis is recognized by the World Organization for Animal Health (OIE) as the world's most widespread zoonosis (BRASIL, 2008). In the Brazilian Northeast, between 2000 and 2015, Pernambuco was the state with the highest morbidity (41.10%) and mortality (10.83%) of humans due to leptospirosis (SANTOS et al., 2018). In Brazil, leptospirosis is a reportable disease under epidemiological surveillance in both humans and animals. According to Hartskeerl et al. (2011), although leptospirosis is cosmopolitan, it is still a neglected disease; several outbreaks have occurred worldwide, with the true extent and incidence of the disease being unknown as surveillance systems are highly variable or often absent.

The World Organization for Animal Health (OIE) considers the Microscopic Agglutination Test (MAT) to be the international standard for the diagnosis of leptospirosis (OIE, 2014). The MAT is able to detect antibodies against several serogroups of *Leptospira* sp. (VIEIRA et al., 2013) having as main advantage its high specificity (BOURHY et al., 2013). According to Pinna (2008), the control and prevention of leptospirosis in equids is dependent on the identification of serogroups that infect equids on the property in order, to classify the disease as accidental or of maintenance.

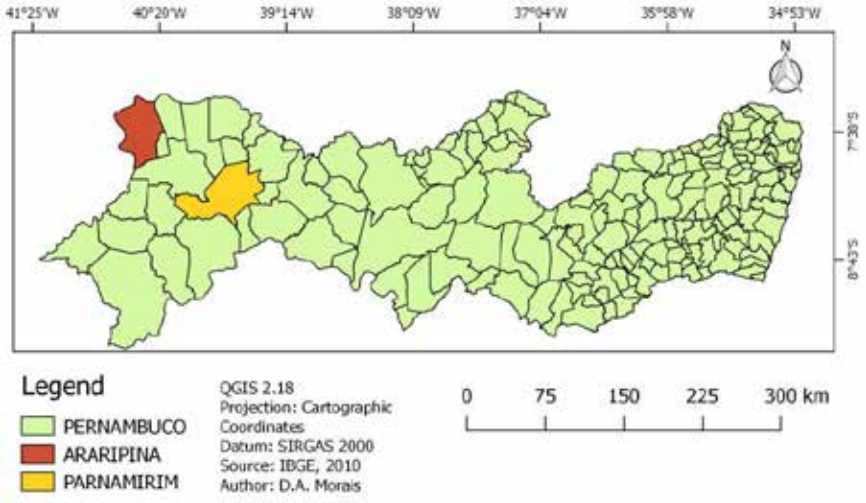
Considering the significant number of donkeys in the Northeast region, the damage that leptospirosis can cause to both the animals and public health is significant; there have been scarce scientific publications on the subject, especially in the caatinga biome. This study aimed at determining the frequency of antibodies to *Leptospira* sp. in the Northeastern Donkeys of the semi-arid region of Brazil destined for slaughter for export.

Material and Methods

Study and sampling area

The research was conducted in September 2017 in two properties (A and B) specializing in get donkeys and send them for slaughter in Amargosa, state of Bahia, Brazil, for subsequent export to China; both properties are located in the mesoregion of the Sertão of Pernambuco (Figure 1), where the BSwh semi-arid tropical climate predominates, according to the classification of Köppen and Geiger (1928), and Caatinga is the predominant vegetation cover (PINHEIRO; ALVES, 2007).

Figure 1. Location of the municipalities of Araripina and Parnamirim in the semi-arid region of Pernambuco, Brazil.



Property A is located in the municipality of Parnamirim, in the Sertaneja Depression, located at latitude 8° 5' 26" South, longitude 39° 34' 42" West, with altitude of 392 m, and area of 2595.92 km², with average temperature and precipitation of 26ºC and 569 mm, respectively. Property B is located in the municipality of Araripina in the extreme northwest portion of the state of Pernambuco, located at latitude 7° 34' 34" South, longitude 40° 29' 54" West, with average altitude of 622 m, and area of 1892.6 km², and average temperature and precipitation of 26°C and 431.8 mm, respectively (MASCARENHAS et al., 2005).

The formula for simple random samples was used to define the minimum number of animals that needed to be sampled (THRUSFIELD, 2007):

$$n=Z^2 \frac{Epre\ (1-Epre)}{d^2}$$

Being:

n = sample size

z = normal distribution value for a 95% confidence level

Epre = expected prevalence

d = statistical error

The parameters used were: expected prevalence of 50% (maximization of the sample), 95% confidence interval and absolute error of 6%. Although the minimum sample number obtained using these parameters was 267 animals, 349 were used, corresponding to the total number of donkeys on the properties. Property A provided 300 donkeys and property B provided 49; there were 147 males and 202 females, aged 3 to 12 years, unvaccinated, all bred in the same environment in an extensive regime, feeding on the native pasture, and having access only to well water.

After the animals were immobilized, blood samples were collected by venipuncture of the external jugular vein using disposable 10 mL syringes and immediately placed in a sterile test tube without anticoagulant. After harvesting, the tubes were duly identified and sent to the Laboratory of Transmissible Diseases (LDT) of the Federal University of Campina Grande (UFCG), where the serum was transferred to microtubes of 1.5 ml and stored at -20ºC until the serological testing was performed.

Diagnostic method

The presence of anti-*Leptospira* sp. antibodies was determined via the MAT method (OIE, 2014) using a collection of 24 strains as antigens: L*eptospira biflexa*: Andamana and Patoc; *Leptospira interrogans*: Australis, Copenhageni, Bataviae, Bratislava, Canicola, Grippotyphosa, Hardjoprajitno, Pomona, Pyrogenes, Icterohaemorrhagiae, Hebdomadis, Wolffi, and Butembo; *Leptospira borgpetersenii*: Autumnalis, Castellonis, Hardjobovis, Javanica, and Tarassovi; *Leptospira santarosai*: Guaricura and Shermani; *Leptospira kirschneri*: Cynopteri; and *Leptospira noguchii*: Panama. These antigen strains were provided by the Laboratory of Veterinary Bacteriology of the Fluminense Federal University (UFF) and originated from the Pasteur Institute, France.

All samples with agglutination activity at the 1:50 dilution were considered positive and were serially titrated at a ratio of two. The antibody titer was the reciprocal with 50% of agglutinations and the highest dilution, and the highest titer in each sample corresponded to the infecting serogroup.

Statistical analysis

The animals were compared for age and sex by association analysis using the chi-square test ($\chi 2$) with a significance level of 5%. The analyses were performed using the R software (R Core Team, 2018).

Results and Discussion

This is the first seroprevalence study for leptospirosis in donkeys bred in the caatinga biome of the semi-arid region of Northeast Brazil. The seropositivity rate found was 19.8% (95% CI = 15.6% - 23.9%) (Table 1); this value is significant considering the rusticity of the species and the adverse conditions of the semi-arid region of Pernambuco, as well as the lower rainfall rate observed in the period and region studied. Sebek et al. (1989) reported similar seropositivity results (17%) in donkeys evaluated in settlements with waste disposal in the periphery of Cairo, Egypt, a location with an arid subtropical climate with virtually no rainfall throughout the year. Therefore, the environmental similarities between the study areas may explain the similar results.

Table 1. Frequency of anti-*Leptospira* sp. antibodies in donkeys by property and respective serogroups in semi-arid donkeys, Northeastern Brazil.

Municipality (Property)	Samples	Positive (%)	Serogroups (n*)
Parnamirim (A)	300	58 (19.3)	Icterohaemorrhagiae (24), Australis (14), Sejroe (6), Pomona (6), Autumnalis (4), Celledoni (2), Tarassovi (2)
Araripina (B)	49	11 (22.4)	Australis (5), Icterohaemorrhagiae (4), Autumnalis (2)
TOTAL	349	69 (19.8)	

*Number of animals with an antibody titer of ≥ 1:50 to *Leptospira* sp. per serogroup.

Higher rates (28.6%) were obtained by Oliveira Filho et al. (2014) when analyzing donkeys in the Brejo Paraíbano; according to Costa et al. (2015), this is a micro-region near the coast with similar characteristics and climatic regime, differing from the semi-arid climate of the municipalities analyzed in this study. Esquivel et al. (2018), obtained a seropositivity rate of 77.8% in donkeys destined for slaughter in Durango, Mexico; the average temperature in this area is below that of the semi-arid region of Pernambuco (17°C) and has considerable humidity, favoring the survival of the agent in the environment.

In this study, a higher rate of seropositivity was observed in donkeys from property B (22.4%) versus property A (19.3%) (Table 1). In 2017, until September, Araripina had a higher rainfall total (606.2 mm), versus 231.6 mm in Parnamirim. This difference is explained by Alves et al. (1996) who established the ideal minimum limit of the rainfall index for the environmental survival of leptospires in the semi-arid region and the maintenance of mechanisms of spread of the disease as being between 500 mm and 550 mm. Moreover, Jung et al. (2010) observed that leptospires eliminated by infected animals may remain infectious for long periods in humid climates, but have reduced opportunity to infect animals in drier locations (ODONTSETSEG et al., 2005).

The positivity rate for leptospirosis obtained in studies on animals destined for traction in large urban centers is higher than that found in our study with animals kept in rural areas. Bezerra et al. (2010) found an 85% seropositivity rate in donkeys from São Luís, Maranhão, and Dias et al. (2015) found a 75% seropositivity rate in equids from Belém, Pará. The high rate found in these studies can be explained by the intense and disordered urbanization associated with the rapid growth of the capitals, lack of basic sanitation, and inadequate management of refuse that provides favorable environmental conditions for the reproduction of rodents, the main reservoirs of the agent (GUIMARÃES et al., 2014).

Lower seropositivity rates (7.7%) were found by Samir et al. (2015) in Egypt. The differences in seropositivity rates can be explained by the several factors that influence the occurrence of leptospirosis, such as topography, region, temperature, humidity, rainfall (ALVES et al., 2000), hygiene and health management of herds, as well as the degree and type of exposure to reservoirs that are known to influence the epidemiology of this disease (LINHARES et al., 2005). Other contributing factors include the existence of clinical suspicion in the animals, the number and types of serogroups used in MAT (BEZERRA et al., 2010), and the interpretation of the results based on the cut-off point used (OLIVEIRA FILHO et al., 2014).

Titers obtained ranged from 50 to 800 (Table 2). It was observed that the rate of positive animals increased from 10.3% to 19.8% when using a cutoff point of 1:50 in MAT. This may be due to the resistance of donkeys to *Leptospira* sp. infection, as described by Shimabukuro et al. (2001), with these animals replicating antibodies at lower levels and thus not being detected at the standard cut-off point (1:100). Additionally, these animals may be chronically infected. Approximately ten days after

initial infection, opsonizing antibodies appeared in the bloodstream; these antibodies promote the gradual elimination of leptospires from the bloodstream, then migrate to immune-protected sites, such as the kidney and genital tract, potentially remaining there for prolonged periods (SIMÕES et al., 2016) and considerably reducing the number of antibodies that can be identified by MAT.

Table 2. Serogroups of *Leptospira* sp. and their titers in semiarid donkeys.

Serogroups	Titers					
	1:50	1:100	1:200	1:400	1:800	TOTAL (%)
Icterohaemorrhagiae	12	11	2	1	2	28 (40.6)
Australis	10	5	2	2	0	19 (27.5)
Autumnalis	4	2	0	0	0	6 (8.7)
Sejroe	2	1	1	1	1	6 (8.7)
Pomona	3	1	1	1	0	6 (8.7)
Celledoni	1	1	0	0	0	2 (2.9)
Tarassovi	1	1	0	0	0	2 (2.9)
TOTAL (%)	33 (47.8)	22 (31.9)	6 (8.7)	5 (7.2)	3 (4.3)	69 (100)

The antibody titer levels detected by MAT depend primarily the level of exposure of a given population to the agent. Thus, the determination of an appropriate cut-off point must consider animals that may be resistant to the disease, as well as the favorability of the region to the survival of the agent (ADLER, 2015). Since the semi-arid region provides adverse environmental conditions for the etiologic agent of leptospirosis, the level of exposure of the analyzed herd to the agent is decreased. The existence of these variables suggests the choice of a cut-off point of 1:50 for MAT, also recommended by Picardeau et al. (2013) and Genovez et al. (2011).

The presence of antibodies against *Leptospira* sp. identified in this study can be explained by the natural exposure of donkeys to sources of infection (HAJIKOLAEI et al., 2005), as they were because they were raised extensively, being kept in an environment that facilitated contact with other species, especially wild animals (BEZERRA et al., 2010). Additionally, these animals have low zootechnical value and, consequently, most of their owners do not offer them proper health care (OLIVEIRA FILHO et al., 2014). Moreover, donkeys destined for slaughter, unlike what occurs in the beef market, are not from equids breeding farms destined for slaughter, and these animals are acquired for this purpose randomly, which hinders the control of their hygiene and health management.

A lower seropositivity rate for *Leptospira* sp. was found in equids kept in stalls when compared to equines partially maintained in stalls or kept primarily on pasture due to increased exposure to infected materials (OLIVEIRA FILHO et al., 2014). Additionally, donkeys were obtained from various locations and subsequently managed in mixed groups until being sent to the slaughterhouse. Lees and Gale (1994) found that individually managed equids were half as likely to be positive for *Leptospira* sp. when compared to those managed in groups.

The *Leptospira* sp. serogroups with seropositivity identified in this study were Icterohaemorrhagiae (40.6%), Australis (27.5%), Autumnalis (8.7%), Sejroe (8.7%), Pomona (8.7%), Celledoni (2.9%), and Tarassovi (2.9%). This diversity may indicate that the donkeys had contact with different species carrying *Leptospira* sp., demonstrating that although donkeys are resistant to the infection, several serogroups are circulating in the species.

The Icterohaemorrhagiae serogroup was identified as the most frequent serogroup in other studies on equids (BEZERRA et al., 2010; DIAS et al., 2015; ESQUIVEL et al., 2018); it is considered the most prevalent serogroup in the species and responsible for most infections in humans (BARBOSA NETA et al., 2016). Infection by this serogroup is associated with the presence of rodents and exposure to contaminated urine from these animals (HAMOND, 2012), facilitated by the extensive breeding of donkeys in this study. The Icterohaemorrhagiae serogroup has already been reported in wild animals (JORGE et al., 2012), and they have been implicated as eliminators of leptospires in the semi-arid region of Brazil (ALVES et al., 2001). The possibility of these animals transmitting leptospirosis to donkeys should not be discarded, as they may be a source of infection and disseminators of the agent in the environment. Therefore, prevention and control measures are essential to break the disease cycle.

The Australis serogroup, with the second most frequent seropositivity in this study, was of noted importance in equids in the studies of Moraes et al. (2010) in Pará, Pires Neto et al. (2005) in the Rio Grande do Sul, Lima et al. (1999) in Paraíba, and Lilenbaum (1998) in Rio de Janeiro, due to its distribution throughout the country and the necessity of its control. Although this serogroup has pigs as maintenance hosts (QUINN et al., 2005), Pinho et al. (2014) argued that they are wild animals. Several authors state that equids are the maintainers of the Australis serogroup (FAINE, 2000; LILENBAUM, 1998; PINNA et al., 2008; RADOSTITS et al., 2000; ROCHA et al., 2004), thus highlighting the importance of the species as a reservoir, acting as a source of infection in the region. Reproductive problems are the most common effect of infection with this serogroup (QUINN et al., 2005), which is typically asymptomatic and impairs the reproductive efficiency of the herd (PINNA, 2008).

It was found in the association analyses that donkeys under nine years of age were more frequently seropositive for *Leptospira* sp. (Table 3). Similar results were found by Moraes et al. (2010) in their study with equids from Algodoal Island, in the state of Pará, where they observed a higher number of seropositive individuals in the younger age groups. This may indicate older animals adapt to the presence of *Leptospira* sp. organisms in the environment by acquiring resistance to infection with age.

Our findings demonstrate the importance of establishing targeted prevention and control measures for leptospirosis in donkeys of the semi-arid region of Pernambuco destined for slaughter, including the improvement of hygiene and monitoring the health status of animals on the management properties. The control of rodents through both elimination and prevention methods and avoiding overcrowding in the pickets by separating by groups according to age are additional important measures to help reduce exposure to *Leptospira* sp. organisms. Finally, control of animal traffic between properties by the Official Veterinary Service aimed at reducing exposure of donkeys to leptospirosis and consequently reducing the transmission of the disease to human beings and animals is an important part of disease prevention.

Table 3. Result of the analysis of the association of age and sex with seropositivity for *Leptospira* sp. in donkeys destined for slaughter in the semi-arid region of Northeastern Brazil.

Variable	Category	Total animals	Positive animals (%)	*P**
Age	3 to 5 years	135	27 (20)	
	6 to 8 years	159	39 (24.5)	
	9 to 12 years	55	3 (5.5)	0.009
Sex	Male	147	27 (18.4)	
	Female	202	42 (20.8)	0.670

*Probability of random occurrence.

More comprehensive studies on this subject in the semi-arid region of Pernambuco, Northeast Brazil, using direct diagnostic tests, such as isolation or Polymerase Chain Reaction (PCR), are necessary to differentiate exposed animals from animals that are infected or acting as carriers. An epidemiological survey aimed at identifying the risk factors is also needed.

Conclusion

The results of this study showed the presence of anti-*Leptospira* sp. antibodies in Northeastern Donkeys from the semi-arid region of Pernambuco, Northeastern Brazil, destined for slaughter and export. This suggests that dissemination of leptospirosis is possible even in resistant animals under semi-arid climate conditions.

Acknowledgments

We thank the National Council for Scientific and Technological Development (CNPQ) for providing us a research grant.

The article was approved by the Research Ethics Committee (CEP) of the Center for Health and Rural Technology of the Federal University of Campina Grande (CSTR/UFCG) under number 085/2017 and was conducted in accordance to the technical standards of biosafety and ethics.

References

ADLER, B. History of leptospirosis and leptospira. *Current Topics in Microbiology and Immunology,* Amsterdan, v. 387, n. 1, p. 79-84, 2015. DOI: 10.1007/978-3-662-45059-8-1

ALVES, C. J.; ANDRADE, J. S. L.; VASCONCELLOS, S. A.; MORAIS, Z. M. Avaliação dos níveis de aglutininas anti-*Leptospira* em cães no município de Patos-PB, Brasil. *Revista Brasileira de Ciência Veterinária,* Rio de Janeiro, v. 7, n. 1, p. 17-21, 2000. DOI: 10.4322/rbcv.2015.168

ALVES, C. J.; VASCONCELLOS, S. A.; CAMARGO, C. R. A.; MORAIS, Z. M. Influência dos fatores ambientais sobre a proporção de caprinos sororeatores para a leptospirose em cinco centros de criação do estado da Paraíba, Brasil. *Arquivos do Instituto Biológico,* São Paulo, v. 63, n. 2, p. 11-18, 1996.

ALVES, C. J.; VASCONCELLOS, S. A.; MORAIS, Z. M.; AZEVEDO, S. S.; SANTOS, F. A.; ANDRADE, J. S. L. Isolamento de Leptospiras patogênicas a partir de tecido renal de roedor silvestre (Galea spixxi) no Semiárido Paraibano, Brasil. *INICIADOS: IX Encontro de Iniciação Científica da UFPB,* João Pessoa, v. 5, n. 1, p. 389-400, 2001.

BARBOSA NETA, E. I.; BRITO NETO, J.; ARAGÃO, C. P. M.; LEITE, A. K. R. M. Leptospirose em equino: Uma revisão. *Revista Brasileira de Higiene e Sanidade Animal,* Fortaleza, v. 10, n. 4, p. 841-857, 2016. DOI: 10.5935/1981-2965.20160069

BATISTA, J. S.; PAIVA, C. C. P. L.; SILVA, J. B.; COSTA, A. M.; CAVALCANTE, P. H.; PRAÇA, L. M.; OLINDA, R. G.; PAIVA, K. A. R. Avaliação de cinco casos de abortamento associado à leptospirose em éguas no Rio Grande do Norte. *Revista Brasileira de Ciência Veterinária*, Rio de Janeiro, v. 22, n. 3-4, p. 165-170, 2015. DOI: 10.4322/rbcv.2016.008

BEZERRA, D. C.; CHAVES, N. P.; GUERRA, P. C.; PEREIRA, H. M.; SANTOS, H. P. Pesquisa de aglutininas anti-*Leptospira* em soros sanguíneos de asininos (*Equus asinus*) e de condutores de veículos de tração animal na cidade de São Luís, MA, Brasil. *Ciência Animal Brasileira,* Goiânia, v. 11, n. 4, p. 931-937, 2010. DOI: 10.5216/cab.v11i4.9233

BOURHY, P.; VRAY, M.; PICARDEAU, M. Evaluation of an in-house ELISA using the intermediate species *Leptospira fainei* for diagnosis of leptospirosis. *Journal of Medical Microbiology,* Edinburgh, v. 62, n. 1, p. 822-827, 2013. DOI: 10.1099/jmm.0.054304-0

BRAGA, J.; HAMOND, C.; MARTINS, G.; ABREU, R. N.; LILENBAUM, W. Ophtalmic alterations in horses with leptospirosis by serovar icterohaemorrhagiae in Rio de Janeiro, Brazil. *Pesquisa Veterinária Brasileira*, Rio de Janeiro, v. 31, n. 2, p. 147-150, 2011. DOI: 10.1590/S0100-736X2011000200009

BRASIL. Ministério da Saúde. Doenças infecciosas e parasitárias: *guia de bolso.* 7. ed. Brasília: Ministério da Saúde, 2008.

CARRIJO JUNIOR, O. A.; MURAD, J. C. B. *Animais de grande porte II*. Brasília: NT Editora, 2016. 192 p.

COSTA, A. S; OLIVEIRA, V. G.; PEREIRA, A. R.; BORGES, P. F.; ARAÚJO, L. S. S. Estudo do clima na região do brejo paraibano utilizando técnicas de séries temporais, para previsão com o modelo Sarima. *Gaia Scientia,* João Pessoa, v. 9, n. 1, p. 127-133, 2015.

DIAS, H. L. T.; SANTOS, W. R. R.; LIMA, P. D. L.; ARAÚJO, C. V.; NEGRÃO, A. M. G.; VASCONCELLOS, S.A. Inquérito sorológico para leptospirose em condutores de carroças e equídeos de tração em Belém, Pará. *Revista de Ciências Agrárias Amazonian Journal of Agricultural and Environmental Sciences*, Belém, v. 58, n. 4, p. 396-401, 2015. DOI: 10.4322/rca.2013

ESQUIVEL, C. A.; ROMERO, A. C.; SALAS, D. R.; FÉLIX, A. O. A.; DOMÍNGUEZ, M. A.; VALENCIA, J. L. O.; ESQUIVEL, A. A.; TINOCO, J. H.; BARBOZA, J. A. Z.; ANGUIANO, L. F. S. Apparently high Leptospira antibody seropositivity in donkeys for slaughter in three municipalities in Durango, Mexico. *Journal of Veterinary Diagnostic Investigation,* Columbia, v. 30, n. 6, p. 929-932, 2018. DOI: 10.1177/1040638718800358

FAINE, S.; ADLER, B.; BOLIM, C.; PEROLAT, P. Leptospira and leptospirosis, *Medical Sciences,* Melbourne, v. 2, n. 1, p. 212-272, 2000. DOI: 10.1016/S0378-1135(01)00319-4

GENOVEZ, M. E.; ESCÓCIO, C.; CASTRO, V.; GABRIEL, F. H. L.; CHIEBAO, D. P.; AZEVEDO, S. S. Fatores de risco associados à infecção pela *Leptospira* spp. sorovar hardjo em rebanhos exclusivos de ovinos e nos consorciados com bovinos. *Arquivos do Instituto Biológico,* São Paulo, v. 78, n. 4, p. 587-592, 2011.

GREVEMEYER, B.; VANDENPLAS, M.; BEIGEL, B.; CHO, E.; WILLINGHAM, A. L.; VERMA, A. Detection of Leptospiral DNA in the Urine of Donkeys on the Caribbean Island of Saint Kitts. *Veterinary Sciences*, Basel, v. 4, n. 1, p. 2-5, 2017. DOI:10.3390/vetsci4010002

GRINDER, M. I.; KRAUSMAN, P. R.; HOFFMAN, R. S. Equus asinus. *Mammalian Species,* Oxford, v. 794, n. 1, p.1-9, 2006. DOI: 10.1644/794.1

GUIMARÃES, R. M.; CRUZ, O. G.; PARREIRA, V. G.; MAZOTO, M. L.; VIEIRA, J. D.; ASMUS, C. I. R. F. Análise temporal da relação entre leptospirose e ocorrência de inundações por chuvas no município do Rio de Janeiro, Brasil, 2007-2012. *Ciência e Saúde Coletiva*, Rio de Janeiro, v. 19, n. 9, p. 3683-3692, 2014. DOI: 10.1590/1413-81232014199.06432014

HAJIKOLAEI, M. R. H.; GORBANPOUR, M.; HAIDARI, M.; ABDOLLAPOUR, G. Comparison of leptospiral infection in the horse and donkey. *Bulletin of the Veterinary Institute in Pulawy,* Varsóvia, v. 49, n. 2, p. 175-178, 2005.

HAMOND, C.; MARTINS, G.; LAWSON, F. R.; MEDEIROS, M. A.; LILENBAUM, W. The role of horses in the transmission of leptospirosis in an urban tropical area. *Epidemiology and Infection,* Cambridge, v. 15, n. 1, p. 1-3, 2012. DOI: 10.1017/S0950268812000416

HARTSKEERL, R. A.; COLLARES-PEREIRA, M.; ELLIS, W. A. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clinical Microbiology and Infection,* Amsterdã, v. 17, n. 4, p. 494- 501, 2011. DOI: 10.1111/j.1469-0691.2011.03474.x

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE. *Produção da Pecuária Municipal*. Rio de Janeiro: IBGE, 2012.

JORGE, S.; HARTLEBEN, C. P.; SEIXAS, F. K.; COIMBRA, M. A.; STARK, C. B.; LARRONDO, A. G.; AMARAL, M. G.; ALBANO, A. P.; MINELLO, L. F.; DELLAGOSTIN, O. A.; BROD, C. S. Leptospira borgpetersenii from free-living white-eared opossum (Didelphis albiventris): *First isolation in Brazil. Acta Tropica,* Amsterdã, v. 124, n. 1, p. 147-151, 2012. DOI: 10.1016/j.actatropica.2012.07.009

JUNG, B. Y.; LEE, K. W.; HA, T. Y. Seroprevalence of *Leptospira* spp. in clinically healthy racing horses in Korea. *Journal of Veterinary Medical Science*, Tóquio, v. 72, n. 1, p. 197- 201, 2010. DOI: 10.1292/jvms.09-0273

KÖPPEN, W.; GEIGER, R. Klimate der Erde**.** *Gotha:* verlag justus perthes. Wall-map 150cmx200cm, 1928.

LAUS, F.; SPATERNA, A.; FAILACE, V.; PAGGI, E.; SERRI, E.; VULLO, C.; CERQUETELLA, M.; TESEI, B. Reference values for hematological and biochemical parameters of mixed breed donkeys (Equus asinus). *Wulfenia Journal*, Klagenfurt, Austria, v. 22, n. 1, p. 294-304, 2015.

LEES, V. W.; GALES, S. P. Titers to *leptospira* species in horses in Alberta. *Canadian Veterinary Journal***,** v. 35, n. 1, p. 636-640, 1994.

LILENBAUM, W. Leptospirosis on animal reproduction: IV. Serological findings in mares from six farms in Rio de Janeiro, Brazil (1993-1996). *Brazilian Journal of Veterinary Research and Animal Science*, São Paulo, v. 35, n. 2, p. 61-63, 1998. DOI: 10.1590/S1413-95961998000200002

LIMA, V. R.; ALVES, C. J.; VASCONCELLOS, S. A.; MORAIS, Z. M.; MOREIRA, J. O. Aglutininas anti-leptospira em jumentos nordestinos criados na microrregião de Patos, Paraíba, Brasil no período de 1996 a 1997. *Revista Brasileira de Ciência Veterinária,* Rio de Janeiro, v. 6, n. 1, p. 5-8, 1999. DOI: 10.4322/rbcv.2015.123

LINHARES, G. F. C.; GIRIO, R. J. S.; LINHARES, D. C. L.; MONDEIRO, L. C.; OLIVEIRA, A. P. Á. Sorovares de *Leptospira interrogans* e respectivas prevalências em cavalos da microrregião de Goiânia, GO. *Ciência Animal Brasileira,* Goiânia, v. 6, n. 4, p. 255-259, 2005. DOI: 10.5216/cab.v6i4.376

LOURO, M. F. C.; DIAS, R. V. C.; SOTOBLANCO, B. Avaliação do fluido peritoneal de asininos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* Minas Gerais, v. 58, n. 1, p. 955-958, 2006. DOI: 10.1590/S0102-09352006000500038

MASCARENHAS, J. C.; BELTRÃO, B. A.; SOUZA JUNIOR, L. C.; GALVÃO, M. J. T. G.; PEREIRA, S. N.; MIRANDA, J. L. F. Serviço Geológico do Brasil Projeto cadastro de fontes de abastecimento por água subterrânea. Diagnóstico do município de Araripina, estado de Pernambuco. Recife: CPRM/PRODEEM, v. 1, n. 1, p. 26, 2005.

MORAES. C. C. G.; KURODA, R. B. S.; PINHO, A. P. V. B.; YWASAK, F.; MENESES, A. M. C.; MARTINS, A. V.; JÚNIOR, J. M. A.; DIAS, H. L. T.; VASCONCELOS, S. A. Pesquisa de anticorpos para sorovares de Leptospira interrogans patogênicas em equídeos criados na ilha de Algodoal, Estado do Pará. *Revista de ciências agrárias,* Belém, v. 53, n. 2, p. 188-194, 2010. DOI: 10.4322/rca.2011.027

ODONTSETSEG, N.; BOLDBAATAR, D.; MWEENE, A. S.; KIDA, H. Serological prevalence of Leptospira interrogans serovar Bratislava in horses in Mongolia. *Veterinary Record,* London, v. 57, n. 1, p. 518, 2005. DOI: 10.1136/vr.157.17.518

OLIVEIRA FILHO, R. B.; MALTA, K. C.; OLIVEIRA, J. M. B.; SANTANA, V. L. A.; HARROP, M. H.V.; STIPP, D.T.; PINHEIRO JUNIOR, J.W. Epidemiological analysis of *Leptospira* spp. infection in equids from the Brejo Paraibano microregion of Brazil. *Journal of Equine Veterinary Science,* Lexington, v. 34, n. 1, p. 407-414, 2014. DOI: 10.1016/j.jevs.2013.08.001

PAIXÃO, A. P.; SANTOS, P.; ALVES, L. M. C.; PEREIRA, H. M.; CARVALHO, H. F.; COSTA FILHO, V. M.; OLIVEIRA, E. A. A.; SOARES, D. M.; BESERRA, P. A. *Leptospira* spp. em bovinos leiteiros do estado do Maranhão, Brasil: frequência, fatores de risco e mapeamento de rebanhos reagentes. *Arquivos do Instituto Biológico,* São Paulo, v. 83, n. 1, p. 1-12, 2016. DOI: 10.1590/1808-1657001022014

PEREIRA, L. G.; REGATIERI, C.; FERRAZ, G. C.; QUEIROZ-NETO, A.; CURI, R. A. Perspectivas do uso de marcadores moleculares no melhoramento genético de eqüinos de corrida da raça Quarto de Milha. *Veterinária e Zootecnia,* Botucatu, v. 22, n. 3, p. 347-369, 2015.

PICARDEAU, M. Diagnosis and epidemiology of leptospirosis. *Médecine et Maladies Infectieuses,* Grenoble, v. 43, n. 1, p. 1-9, 2013. DOI: 10.1016/j.medmal.2012.11.005

PINHEIRO, K.; ALVES, M. Espécies arbóreas de uma área de Caatinga no sertão de Pernambuco, Brasil: dados preliminares. *Revista Brasileira de Biociências*, Porto Alegre, v. 5, n. 2, p. 426-428, 2007.

PINHO, A. P. V. B.; KURODA, R. B. S.; VASCONCELLOS, S.; FERREIRA NETO, J. S.; OSSADA, R.; SOUZA, V. A. F.; ROCHA, K. S. Serological study of brucellosis and leptospirosis in equines of island Maiandeua (Algodoal) state of Pará. *Semina: Ciências Agrárias*, Londrina, v. 35, n. 6, p. 3221-3230, 2014. DOI: 10.5433/1679-0359.2014

PINNA, M. H.; VARGES, R.; LILENBAUM, W. Aplicação de um programa integrado de controle da leptospirose em equinos no Rio de Janeiro, Brasil. *Revista Brasileira de Ciência Veterinária*, Rio de Janeiro, v. 15, n. 2, p. 63-66, 2008. DOI: 10.4322/rbcv.2014.199

PIRES NETO, J. A. S.; HESSE, F.; OLIVEIRA, M. A. M. Leptospirose equina: aspectos clínicos, tratamento, prevenção e levantamento sorológico. *Veterinária em Foco,* Canoas, v. 2, n. 2, p. 165-176, 2005.

QUINN, P. J.; MARKEY, B.; CARTER, M. E.; DONNELLY, W. J.; LEONARD, F. C. *Microbiologia veterinária e doenças infecciosas.* Porto Alegre: Artmed, 2005. 512 p.

R CORE TEAM. *R:* a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2018.

RADOSTITS, O. M.; GAY, C. C.; BLOOD, D. C.; HINCHILIFF, K. W. *Veterinary Medicine***,** London, v. 9, n. 1, p. 1731-1877, 2000.

ROCHA, T.; ELLIS, W. A.; MONTGOMERY, J.; GILMORE, C.; REGALLA, J.; BREM, S. Microbiological and serological study of leptospirosis in horses at slaughter: first isolations**.** *Veterinary Science,* Curitiba, v. 76, n. 1, p. 1990-202, 2004. DOI: 10.1016/j.rvsc.2003.12.003

SALLES, P. A.; SOUSA, L. O.; GOMES, L. P. B.; BARBOSA, V. V.; MEDEIROS, G. R.; SOUSA, C. M.; WELLER, M. Analysis of the population of equidae in semiarid region of Paraíba. *Journal of Biotechnology and Biodiversity*, Gurupi, v. 4, n .3, p. 269-275, 2013.

SAMIR, A.; SOLIMAN, R.; EL-HARIRI, M.; ABDEL-MOEIN, K.; HATEM, M. E. Leptospirosis in animals and human contacts in Egypt: broad range surveillance. *Revista da Sociedade Brasileira de Medicina*

Tropical, Uberaba, v. 48, n. 3, p. 272-277, 2015. DOI: 10.1590/0037-8682-0102-2015

SANTOS, C. S.; GUEDES JÚNIOR, D. S.; PEREIRA, R. C. G.; SANTOS, C. C. A.; CASTRO, V.; JESUS, V. L. T. Inquérito sorológico da ocorrência de leptospirose em equídeos da microrregião de Itaguaí no estado do Rio de Janeiro-RJ. *Revista Brasileira de Medicina Veterinária*, Rio de Janeiro, v. 34, n. 2, p. 96-100, 2012. DOI: 10.2430/2527.2179

SANTOS, R. F.; SILVA, G. C. P.; ASSIS, N. A.; MATHIAS, L. A. Aglutininas anti- *Leptospira* spp. em equídeos da região sul do Brasil abatidos em matadouro-frigorífico. *Semina: Ciências Agrárias*, Londrina, v. 37, n. 2, p. 841-852, 2016. DOI: 10.5433/1679-0359.2016

SANTOS, Y. S.; SILVA, E. B.; OLIVEIRA JÚNIOR, J. F.; SANTOS, P. J.; COSTA, L. M. B. Diagnóstico da morbidade e mortalidade dos casos de Leptospirose no Nordeste brasileiro entre 2000 a 2015. *Enciclopédia Biosfera, Centro Científico Conhecer,* Goiânia, v. 15, n. 27, p. 107-118, 2018. DOI: 10.18677/EnciBio_2018A34

SEBEK, Z.; SIXL, W.; VALOVA, M.; SCHAFFLER, R. Leptospirosis in man, in wild and in domestic animals at waste disposal sites in Cairo. *Geographia Medica. Supplement*, Budapest, v. 3, n. 1, p.141-150, 1989.

SHIMABUKURO, F. H.; MORAES-SILVA, E.; MENDONÇA, A. O.; CERQUEIRA, E. J. L.; ARAÚJO, W. N.; SARKIS, D. T. Aspectos soroepidemiológicos da leptospirose em equídeos, dos municípios de Jacobina e Jequié, Bahia. *Ciência Veterinária nos Trópicos*, Recife, v. 4, n. 1, p. 274-80, 2001.

SIMÕES, L. S.; SASAHARA, T. H. C.; FAVARON, P. O.; MIGLINO, M. A. Leptospirose -revisão. *Publicações em Medicina Veterinária e Zootecnia,* Maringá, v. 10, n. 2, p. 138-146, 2016. DOI: 10.22256/pubvet.v10n2.138-146.

THRUSFIELD, M. Veterinary epidemiology. *Blackwell Science*, Oxford, v. 3, n. 1, p. 233-610, 2007.

ULIANA, I. C. S.; COSTA, B. H. V.; BIAVA, J. S. Avaliação da qualidade química do leite de jumentas da raça Pêga. *Revista Acadêmica Ciência Animal,* Curitiba, v. 14, n. 1, p. 245-255. DOI: 10.7213/academica.14.2016.27

VIEIRA, A. S. Identificação de mamíferos silvestres do Pantanal Sul-Mato-Grossense portadores de *Leptospira* spp. *Ciência Animal Brasileira,* Goiânia, v. 14, n. 3, p. 373-380, 2013. DOI: 10.5216/cab.v14i3.17147

WORLD ORGANIZATION FOR ANIMAL HEALTH - OIE. Manual of diagnostic Tests and vacines for terrestrial animals. Paris: OIE, 2014.