UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
CENTRO DE SAÚDE E TECNOLOGIA RURAL
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA
CAMPUS DE PATOS

Arthur Willian de Lima Brasil

Levantamento epidemiológico de patógenos de importância em saúde única em cães atendidos em clínicas veterinárias de João Pessoa, Paraíba, nordeste do Brasil

PATOS PB

2017

Arthur Willian de Lima Brasil

Levantamento epidemiológico de patógenos de importância em saúde única em cães atendidos em clínicas veterinárias de João Pessoa, Paraíba, nordeste do Brasil

Tese apresentada ao Programa de Pós-Graduação em Medicina Veterinária do Centro de Saúde e Tecnologia Rural da Universidade Federal de Campina Grande, como parte dos requisitos para obtenção do título de Doutor em Medicina Veterinária.

PROF. DR. SÉRGIO SANTOS DE AZEVEDO

Orientador

PATOS PB

2017

B83l Brasil, Arthur Willian de Lima

Levantamento epidemiológico de patógenos de importância em saúde única em cães atendidos em clínicas veterinárias de João Pessoa, Paraíba / Arthur Willian de Lima Brasil. – João Pessoa, 2017.

60f.; il.

Orientador: Prof. Dr. Sérgio Santos de Azevedo.

Tese (Doutorado em Medicina Veterinária) – Universidade Federal de Campina Grande - UFCG

1. Epidemiologia. 2. Cães Domiciliados. 3. Zoonoses. 4. Controle. 5. Saúde Única. I. Título.

UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
CENTRO DE SAÚDE E TECNOLOGIA RURAL
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA
CAMPUS DE PATOS

Arthur Willian de Lima Brasil

Tese apresentada ao Programa de Pós-Graduação em Medicina Veterinária do Centro de Saúde e Tecnologia Rural da Universidade Federal de Campina Grande, como parte dos requisitos para obtenção do título de Doutor em Medicina Veterinária.

APROVADO 15/12/2017

EXAMINADORES:

Prof. Dr. Sérgio Santos de Azevedo
Presidente

Prof.(a) Dra. Carolina de Sousa Américo Batista Santos
Membro Interno

Prof. Dr. Severino Silvano Dos Santos Higino
Membro Interno

Prof. Dr. Vinicius Longo Ribeiro Vilela
Membro Interno

Prof(a) Dra. Thaís Ferreira Feitosa
Membro Interno

AGRADECIMENTOS

Em primeiro lugar agradeço a Deus, pelo dom da vida e por me fazer persistir para realização desse sonho.

A minha esposa Roberta Nunes Parentoni, meus pais Brasil e Maria Ziza e meus irmãos André e Carol que sempre acreditaram em mim e me deram forças para continuar mesmo nas situações onde não havia saída. Amo vocês.

Ao meu orientador, prof. Dr. Sérgio Santos Azevedo, pela confiança depositada em mim desde a Iniciação Científica, por todos os ensinamentos e oportunidades.

A todos os 34 médicos veterinários que permitiram que eu fizesse esse trabalho em suas clínicas sempre de forma solícita e agradável.

Aos amigos Julierme Ferreira Rocha, Eduardo Dias Ribeiro, George Borja, Rodrigo Alves, Barbara Monteiro, Atticus Tanikawa que sempre foram excelentes conselheiros para todas as horas e que me fizeram sorrir mesmo em momentos difíceis.

Aos amigos de hoje e de sempre Vinicius Vilela, Thais Feitosa, Carolina Américo, Diego Figueiredo, que foram braços fortes para me ajudar e aconseilhar.

Aos amigos da Lucas (Fofo), Álef (Xopan), Augusto (Liberato), Luis Felipe (Inútil), Mauricio (Bowser) e Mamedes que me acolheram na república bico do corvo e que me ajudaram em Botucatu, nunca me esquecerei de vocês!!!

Aos amigos que conquistei durante esse período Lena, Laudy, Elonir, Eloise, Caio, Dal, Elizabeth, Marcelo, Ricardo, Izadora, Nicole, Maria, Everaldo vocês são demais.

Aos colegas do Laboratório de Doenças Transmissíveis especialmente Carla, Camila, Leíse, Davidianne, Devede, Maira, Anniele, Areano e dona Francinete por toda a ajuda prestada e pelos bons momentos de convivência.

Ao professor Dr. Clebert José Alves que sempre me ajudou de todas as situações.

À professora Drª Marcia Almeida Melo do Laboratório de Biologia Molecular do Semi-árido e sua orientada Raizza Barros por toda atenção e prestatividade.

Ao prof. Dr. Hélio Langoni e toda sua equipe do Núcleo de Pesquisa em Zoonoses Noelle, Dália, Mari, Giullia Karine, Carol por toda a acolhida e ensinamentos.

Ao prof. Dr. Rinaldo Mota e sua equipe do Laboratório de Doenças Infectocontagiosas dos Animais Domésticos especialmente seu orientado Givanildo por toda a amizade e conhecimentos compartilhados.

EPÍGRAFE

"*Navegar é preciso, viver não é preciso!*"

(Fernando Pessoa)

RESUMO

O contato entre humanos e animais de estimação tem se intensificado cada vez mais, o que pode representar um risco eminente na transmissão de zoonoses. Essas enfermidades geralmente possuem um forte componente social e ambiental, de maneira que abordagens multissistêmicas e multidisciplinares são necessárias a fim de reduzir a sua ocorrência. Neste contexto, o objetivo desta tese, composta por três capítulos, foi determinar as prevalências e os fatores de risco associados as infecções por *Leptospira* sp., *Toxoplasma gondii*, *Neospora caninum*, *Leishmania* spp. e *Trypanosoma cruzi* em cães atendidos em clínicas veterinárias na cidade de João Pessoa, Paraíba, Nordeste do Brasil. Foram utilizados 384 cães provenientes de 34 clinicas veterinárias no período de abril de 2015 a maio de 2016. Foram determinadas as prevalência de 11,7%, 9,63%, 1,5%, 2,8% e 1,5% para as infecções por *Leptospira* sp., *Toxoplasma gondii*, *Neospora caninum*, *Leishmania* spp. e *Trypanosoma cruzi* respectivamente. Para a infecção por *Leptospira* sp., os fatores de risco foram idade entre 49 e 72 meses (OR = 2,74; IC 95% = 1,01 - 6,05), idade > 72 meses (OR = 3,22; IC 95% = 1,55 – 6,68), e limpeza mensal do ambiente (OR = 10,70; IC 95% = 1,21 – 95,53); para *T. gondii*, os fatores de risco foram acesso à rua (OR = 4,60; IC 95% = 1,73-12,19) e viver em ambiente próximo a matas (OR = 2,79; IC 95% = 1,31-5,93); acesso à rua (OR = 4,81; IC 95% = 1,23 - 18,75) foi identificado como fator de risco associado à infecção por *Leishmania* spp.. Não foram identificados fatores de risco para *N. caninum* e *T. cruzi*. Conclui-se que cães atendidos em clínicas veterinárias de João Pessoa, Paraíba, Nordeste do Brasil, estão expostos às infecções *Leptospira* sp., *T. gondii*, *N. caninum*, *Leishmania* spp. e *T. cruzi*, determinadas por sorologia, bem como sugere-se a condução de medidas de controle baseadas na correção dos fatores de risco identificados.

PALAVRAS-CHAVE: Cães domiciliados; controle; epidemiologia; saúde única; zoonoses.

ABSTRACT

The contact between humans and pets has intensified more and more, which may pose an imminent risk in the transmission of zoonoses. These diseases usually have a strong social and environmental component, so that multisystem and multidisciplinary approaches are necessary to reduce their occurrence. In this context, the objective of this thesis, which consisted of three chapters, was to determine the prevalence and risk factors associated with *Leptospira* sp., *Toxoplasma gondii*, *Neospora caninum*, *Leishmania* spp. and *Trypanosoma cruzi* in dogs attended at veterinary clinics in the city of João Pessoa, Paraíba, Northeast Brazil. A total of 384 dogs were used from 34 veterinary clinics from April 2015 to May 2016. The prevalence of 11.7%, 9.63%, 1.5%, 2.8% and 1.5% for *Leptospira* sp., *Toxoplasma gondii*, *Neospora caninum*, *Leishmania* spp. and *Trypanosoma cruzi* respectively. For the infection by *Leptospira* sp., the risk factors age from 49 to 72 months (odds ratio = 2.74), age > 72 months (odds ratio = 3.22), and monthly cleaning of the environment (odds ratio = 10.70). for *T.* gondii the risk factors for access to the streets (OR = 4.60; 95% CI = 1.74-12.20) and environments close to forested areas (OR = 2.79; 95% CI = 1.32-5.93).; Access to the street (OR = 4.81; 95% CI -18.75 = 1.23) was identified as a risk factor associated with infection by *Leishmania* spp.. No risk factors for *N. caninum* and *T. cruzi* were identified. It is concluded that dogs attended at veterinary clinics in João Pessoa, Paraíba, Northeast Brazil, are exposed to infections *Leptospira* sp., *T. gondii*, *N. caninum*, *Leishmania* spp. and *T. cruzi*, determined by serology, and it is suggested to conduct control measures based on the correction of identified risk factors.

KEYWORDS: Domiciled dog; control; epidemiology; one health; zoonoses.

LISTA DE ILUSTRAÇÕES

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

LISTA DE TABELAS

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

SUMÁRIO

1. INTRODUÇÃO E JUSTIFICATIVA

Os cães foram os primeiros animais domesticados pelos humanos e seu relacionamento data de cerca de 10.000 anos atrás. Dessa relação simbiótica inúmeros benefícios foram observados como proteção, auxílio no trabalho além de companhia e lazer (WANG e TEDFORD, 2008). Nas ultimas décadas tem sido observado um grande apelo ao bem estar animal que expressa melhorias na qualidade de vida, sobretudo no que diz respeito à saúde (BROOM, 2005). Por outro lado, esta proximidade pode apresentar desvantagens principalmente relacionadas à transmissão de agentes zoonóticos.

A ocorrência de zoonoses está relacionada a desequilíbrios ambientais, como desmatamento e urbanização, a fatores socioeconômicos, como pobreza e marginalização e a fatores culturais, como hábitos vida. Sendo assim, a utilização dos conceitos de saúde única (*One Health,* abordagem integrada que reconhece a interconectividade entre a saúde humana, dos demais seres vivos e do a do ambiente) são essenciais, uma vez que eles proporcionam abordagens complexas e multisistêmicas que avaliam através de diversos pontos de vista as enfermidades. Diante disto, doenças como a leptospirose, toxoplasmose, leishmaniose, doença de chagas e neosporose, que são influenciadas por fortes componentes ambientais e sociais, devem ser continuamente monitoradas e avaliados seus riscos de transmissão (ACHA e SZYFERES, 2001).

A leptospirose é uma enfermidade causada por *Leptospira* sp. que possui duas formas de apresentação, uma assintomática que esta relacionada a hospedeiros adaptados os quais normalmente não apresentam sinais clínicos e eliminam o agente na urina e a outra de caráter clínico agudo e grave atrelada a hospedeiros acidentais (ADLER e MOTECZUMA, 2010).

A toxoplasmose é causada por *Toxoplasma gondii*, protozoário intracelular obrigatório. É reconhecida por ser uma doença oportunista relacionada com a baixa imunidade e geralmente é assintomática. Apesar dos cães não serem hospedeiros definitivos, podem contribuir na disseminação do parasita de forma mecânica (FRENKEL et al. 2003).

A neosporose é uma doença grave que afeta principalmente cães e bovinos causando alterações neurológicas e reprodutivas, respectivamente. É causada por *Neospora caninum* e possui como hospedeiros definitivos os cães (DUBEY, 2003), que

eliminam as formas infectantes (oocistos) no ambiente através das suas fezes. Embora a neosporose não tenha o status de zoonose existem indícios sorológicos que os humanos apresentam anticorpos para o protozoário, principalmente pessoas imunocomprometidas (OSHIRO et al., 2015).

A leishmaniose visceral e a doença de Chagas são protozooses causada por leishmanias do complexo Donovani e o *Trypanossoma cruzi*, respectivamente (GONTIJO e MELO, 2004; DIAS, 2002). Os cães são os principais reservatórios dos parasitas e ambos são transmitidos por vetores. Essas enfermidades são consideradas endêmicas no Brasil, sendo causadoras de óbitos entre humanos e animais (BRASIL, 2006; BRASIL, 2010).

A transmissão dessas enfermidades tem em comum o contato próximo com cães sendo necessária a realização de monitoramento da sanidade dos animais a fim de controlar a disseminação dos patógenos. Além disso, os estudos epidemiológicos sobre agentes zoonóticos fornecem indicadores para que sejam realizadas politicas públicas a fim de reduzir o número de casos positivos.

A presente tese de doutorado é composta por três capítulos constituídos por artigos científicos originais. O Capítulo I é consiste a um artigo científico submetido à Revista Semina Ciências Agrárias - Qualis B1, e descreve as soroprevalências e os fatores de risco para a infecção por *Leptospira* sp. em cães atendidos em clínicas veterinárias de João Pessoa, Estado da Paraíba, no Nordeste brasileiro. O Capítulo II é composto por um artigo submetido à Revista Brasileira de Parasitologia Veterinária - Qualis A2, no qual foram avaliadas as soroprevalências e os fatores de risco para as infecções por *Toxoplasma gondii* e *Neospora caninum* em cães atendidos em clínicas veterinárias de João Pessoa Estado da Paraíba, Nordeste do Brasil. O Capítulo III compreende um artigo científico submetido à Revista Semina Ciências Agrárias - Qualis B1, na qual foi investigada a prevalência *Leishmania* spp., *T. cruzi*,. em cães atendidos em clínicas veterinárias de João Pessoa Estado da Paraíba, Nordeste do Brasil.

2. REFERÊNCIAS

ACHA, P.N.; SZYFRES, B. *Zoonosis y enfermidades transmissibles comunes al hombre y a los animales.* Washington: Organizacion Panamericana de La Salud. 3 ed., 2001. 580 p.

ADLER, B; MOCTEZUMA, A.P. Leptospira and leptospirosis. *Veterinary Microbiology*. Amisterdam, v.27, n.140 (3-4), p.287-296, 2010.

BRASIL. *Manual de vigilância e controle da leishmaniose visceral.* Brasília: Editora do Ministério da Saúde, 2006. 120 p.

BRASIL. *Doenças infecciosas e parasitárias: guia de bolso*. 8. ed. Brasília: Ministério da Saúde, 2010. 448 p

BROOM, D.M. Animal welfare education: development and prospects. *Journal Veterinary Medicine Education.* Toronto, v 32, p.438 – 441, 2005.

DIAS J.C.; SILVEIRA A.C.; SCHOFIELD C.J. The impact of Chagas disease control in Latin America: a review. *Memórias Instituto Oswaldo Cruz*, Rio de Janeiro v. 97, n.5, p.603-612, 2002.

DUBEY, J.P. Review of *Neospora caninum* and neosporosis in animals. *The Korean Journal of Parasitology*, Seul, v.41, n.1, p:1-16, 2003.

GONTIJO, C.M.F.; MELO, M. N. Leishmaniose visceral no Brasil. *Revista Brasileira de Epidemiologia,* São Paulo, v. 7, n. 3, p. 338-349, 2004.

FRENKEL, J.K.; LINDSAY, D.S.; PARKER, B.B.; DOBESH, M. Dogs as possible mechanical carriers of Toxoplasma, and their fur as a source of infection of young children. *International Journal of Infectious Diseases*, v. 7, n.1, p:292-293, 2003.

OSHIRO, L. M.; et al. *Neospora caninum* and *Toxoplasma gondii* serodiagnosis in human immunodeficiency virus carriers. Revista da Sociedade Brasileira Medicina Tropical, Uberaba, v. 48, n. 5, p. 568-572, 2015 .

WANG, X. TEDFORD, R.H. *Dogs: Their Fossil Relatives and Evolutionary History*. Nova Iorque,: Columbia University Press, 2008. 209p.

3. CAPITULO I

PREVALENCE OF AND RISK FACTORS TO *Leptospira* spp. INFECTION IN DOGS ATTENDED AT VETERINARY CLINICS IN JOÃO PESSOA, PARAÍBA STATE, NORTHEASTERN BRAZIL

"Prevalência e fatores de risco da infecção por *Leptospira* spp. em cães atendidos em clínicas veterinárias em João Pessoa, Paraíba, Nordeste Brasil"

(Artigo submetido à revista Semina: Ciências Agrárias, Qualis B1)

Prevalence of and risk factors to *Leptospira* spp. infection in dogs attended at veterinary clinics in João Pessoa, Paraíba State, Northeastern Brazil

Prevalência e fatores de risco da infecção por *Leptospira* spp. em cães atendidos em clínicas veterinárias em João Pessoa, Paraíba, Nordeste Brasil

Arthur Willian de Lima Brasil[1]; Diego Figueiredo da Costa[1]; Carla Lauise Rodrigues Menezes Pimenta[1]; Roberta Nunes Parentoni[1]; Clebert José Alves[1]; Sérgio Santos de Azevedo[1]

[1]Center of Rural Health and Technology, Federal University of Campina Grande (UFCG), Patos, PB, Brazil. E-mail: arthurwillian7@yahoo.com.br; roberta.parentoni@gmail.com; diegoveter@hotmail.com; carlalauise@hotmail.com; clebertja@uol.com.br; sergio@vps.fmvz.ups.br

Abstract

The aim of this study was to determine the prevalence and risk factors associated with *Leptospira* sp. in dogs attended at veterinary clinics in the city of João Pessoa, State of Paraíba, Northeast Brazil. A total of 384 blood samples from dogs from 34 veterinary clinics were used from April 2015 to May 2016. The diagnosis of *Leptospira* sp. was carried out through Microscopic Agglutination Test (MAT), using a collection of 20 pathogenic antigens and adopting a 1: 100 dilution as cutoff point. An epidemiological questionnaire was applied to the owners of the animals to obtain data to be used in the analysis of risk factors. The prevalence of seropositive animals was 11.7% (45/384), with reactions for serogroups Icterohaemorrhagiae (62.3%), Grippotyphosa (22.2%), Canicola (13.3%), Djasiman 2%) and Pomona (2.2%). The following risk factors were identified: age from 49 to 72 months (odds ratio = 2.74), Age > 72 months (odds ratio = 3.22), and monthly cleaning of the environment where the animals are kept (odds ratio = 10,70). It is concluded that dogs attended at veterinary clinics in João Pessoa are exposed to infection by *Leptospira* sp., with predominance of serogroups kept by wild animals, and it is suggested that the periodic cleaning of the environment where the animals are kept.

Key words: Canine leptospirosis, Microscopic agglutination test, Epidemiology, Control.

Resumo

O objetivo deste estudo foi determinar a prevalência e os fatores de risco associados à infecção por *Leptospira* sp. em cães atendidos em clínicas veterinárias da cidade de João Pessoa, Estado da Paraíba, Nordeste do Brasil. Foram utilizadas 384 amostras sanguíneas de cães provenientes

de 34 clínicas veterinárias no período de abril de 2015 a maio de 2016. O diagnóstico da infecção por *Leptospira* sp. foi realizado através da Soroaglutinação Microscópica (SAM), utilizando uma coleção com 20 antígenos patogênicos e adotando como ponto de corte a diluição 1:100. Foi aplicado um questionário epidemiológico aos proprietários dos animais para obtenção de dados a serem utilizados na análise de fatores de risco. A prevalência de animais soropositivos foi de 11,7% (45/384), com reações para os sorogrupos Icterohaemorrhagiae (62,3%), Grippotyphosa (22,2%), Canicola (13,3%), Djasiman (2,2%) e Pomona (2,2%). Foram identificados os seguintes fatores de risco: idade entre 49 e 72 meses (*odds ratio* = 2,74), idade maior que 72 meses (*odds ratio* = 3,22), e limpeza mensal do ambiente onde os animais permanecem (*odds ratio* = 10,70). Conclui-se que cães atendidos em clínicas veterinárias de João Pessoa estão expostos à infecção por *Leptospira* sp., com predominância de sorogrupos mantidos por animais selvagens, bem como sugere-se que seja realizada a limpeza periódica do ambiente onde os animais permanecem.

Palavras-chave: Leptospirose canina, Soroaglutinação microscópica, Epidemiologia, Controle.

Introduction

The relationship among humans, animals and the environment has generated severe ecological imbalances, causing the depletion of natural resources. These environmental changes directly affect the dynamics of zoonoses, changing their forms of presentation, occurrence and pathogenicity (MWANGI et al., 2016). In this context, pets, such as dogs, which are increasingly incorporated into family nuclei as members of the family, may play a major role in the maintenance and transmission of infectious agents, besides being sentinels for the respective diseases (ULLMAN et al., 2008). In this way, diseases such as leptospirosis need to be monitored and their risks assessed in the ecosystem.

Leptospirosis is caused by bacteria of the genus *Leptospira*, classified according to homology of DNA in three groups: pathogenic, with 10 species; intermediates, five species; saprophytic, six species (BOURHY et al., 2014). Disease transmission occurs by direct contact of the intact or damaged skin and mucous membrane with urine, and by indirect contact with contaminated water (HAAKE; LEVETT, 2014).

Pathogenic *Leptospira* sp. may cause reproductive, renal and liver disorders or even unapparent infection, depending on the infecting serovar. Serovars adapted to dogs generally cause chronic infection with absence of clinical signs. However, the manifestation is generally acute and severe in infections caused by accidental serovars (ADLER; MOCTEZUMA, 2010).

Rodents are potential reservoirs of leptospires, and may eliminate them through the urine (FAINE et al., 1999). However, due to the closeness with human beings, dogs play an

important role in the transmission of leptospires, as they may harbor the agent in the kidneys and eliminate it in the urine in a continuous or intermittent form (MIOTO et al., 2016).

Moreover, the municipality of João Pessoa reported an occurrence of 86 cases of human leptospirosis between 2007 and 2015 (SINAN, 2017), which reinforces the need to conduct epidemiological surveys in dogs once these animals are important source of infection of *Leptospira* sp. in urban environment. Therefore, this survey aimed to determine the prevalence of *Leptospira* sp. infection in dogs attended at veterinary clinics of João Pessoa, Paraíba state, Northeastern Brazil, as well as to identify risk factors associated with the infection.

Material and Methods

Study area

The survey was conducted in the municipality of João Pessoa, capital city of the state of Paraíba, which has about 720 thousand inhabitants (BRASIL, 2010), distributed in 59 boroughs, and with an area of preserved Atlantic Forest in the center of the urban spot and bordered by mangroves (PARAÍBA, 2014). Its climate is humid tropical, with annual average temperatures of 23ºC and annual rainfall rate above 1,906 mm (PEREIRA, 2014).

Sampling

The selection of the veterinary clinics was based on data from the Regional Council of Veterinary Medicine (CRMV-PB), which has informed the existence of 40 registered clinics, however, in the on-the-spot verification four establishments had closed and in two others, the owners preferred not to participate. Then, 34 veterinary clinics in the municipality of João Pessoa distributed in the four zones of the city (north, south, east and west) were used.

The minimum number of animals was determined by the formula for simple random sampling (THRUSFIELD, 2004):

$$n = \frac{Z^2 \text{ x } P\ (1 - P)}{d^2}$$

Where:

n = number of sampled animals

Z = value of the normal distribution for the confidence level of 95%

P = expected prevalence of 50% (sampling maximization)

d = error of 5%

In total, 384 apparently healthy dogs, unvaccinated or vaccinated over six months against leptospirosis were selected. Females in the periparturient and lactation periods were

excluded from the study. The collections were carried out in the period from April 2015 to May 2016. Samples were collected by venipuncture of the cephalic or jugular vein, using vacuum tubes of 5 mL, and sera were placed into microtubes and frozen until the serologic tests were performed.

Serologic diagnosis of Leptospira sp. infection

The serologic diagnosis was performed by the microscopic agglutination technique (MAT) (GALTON et al., 1965; COLE et al., 1973), which is the test recommended by the World Organization for Animal Health (OIE). A collection of 20 serovars of *Leptospira* sp. were used: Australis, Copenhageni, Bataviae, Bratislava, Canicola, Grippotyphosa, Hardjoprajitno, Pomona, Icterohaemorrhagiae, Hebdomadis, Wolffi, Autumnalis, Castellonis, Hardjobovis, Hardjoprajitno, Tarassovi, Sejroe, Guaricura, Cynopteri and Panama, provided by the Veterinary Bacteriology Laboratory of the Fluminense Federal University (UFF) and originated from the Pasteur Institute, France. Reactive samples at dilution rate 1:100 needed to be diluted two folds to determine the maximum positive dilution with 50% agglutination. The probable serogroup for each sample was the one that showed the highest titration, and the remaining agglutinations were considered cross-reactions. Animals which had two or more serogroups with identical titration were positive, and considered for the prevalence calculation, although not considered for calculating the most common serogroups (OIE, 2014).

Risk factor analysis

Epidemiological questionnaires were applied to dog owners in order to obtain data to be used in the risk factor analysis. The analyzed variables and their respective categories were: educational level of the owner (illiterate, incomplete primary school, complete primary school, incomplete high school, complete high school, incomplete university degree, university degree), gender (female, male), breed (pure-bred, crossbred), age (up to 48 months, 49 to 72 months, above 72 months), access to street (no, yes), feed (commercial food, homemade food, food scraps, raw meat), access to treated water (no, yes), reason for keeping the dog (company, guard, other), contact with dogs (no, yes), contact with bovine (no, yes), contact with equine (no, yes), contact with wild animals (no, yes), contact with cats (no, yes), contact with goats (no, yes), contact with sheep (no, yes), contact with swine (no, yes), type of housing (masonry, shacks, stilts, mud huts), environment where the animal lives (soil, cement, soil/cement), house near woodlands or agricultural areas (no, yes), regular garbage collection (no, yes), cleaning and disinfection of the environment where the animals are kept (daily, weekly/fortnightly, monthly), vaccination (no, yes), contact with flooded areas (no, yes), presence of rodents (no,

yes), occurrence of abortions (no, yes), occurrence of stillbirths (no, yes), presence of ticks (no, yes).

An univariable exploratory analysis of the data was carried out for the selection of variables with $P \leq 0.2$ using the chi-square or Fischer exact tests. Subsequently the significant variables passed to a multivariable analysis using the multiple logistic regression with significant level of 5% (HOSMER; LEMESHOW, 2000). The adjustment of the final model was verified with the Hosmer and Lemeshow test, by which a value of $P \geq 0.05$ indicates a good fit. The collinearity between independent variables was verified by correlation analysis; for the variables with strong collinearity (correlation coefficient > 0.9), one of the two variables was excluded from the multiple analysis according to the biological plausibility (DOHOO et al., 1997). Confounding was evaluated by the monitoring the alterations in the model's parameters (> 20%) when adding new variables. Data analyses were carried out using the SPSS 23.0 *for* Windows software.

Results and Discussion

Figure 1 presents the geographical distribution of the dogs attended at the veterinary clinics according to the zone. All animals were proceeding from the city of João Pessoa. Of the 384 evaluated samples 45 were seropositive, with a prevalence of 11.7%. The most frequent serogroup was Icterohaemorrhagiae (62.3%), followed by Grippotyphosa (22.2%), Canicola (13.3%), Djasmani (2.2%) and Pomona (2.2%), with antibody titers ranging from 100 to 3200 (Table 1).

The prevalence of *Leptospira* sp. positive dogs attended at veterinary clinics in João Pessoa wera larger than those to that found in Natal, Rio Grande do Norte state, where seropositivity in dogs from veterinary clinics was 6.8% (FERNANDES et al., 2013). Other studies on the presence of anti-*Leptospira* sp. antibodies in domiciled and stray dogs in Paraíba state referred frequencies of 19.7% and 20.4% (BATISTA et al., 2004; AZEVEDO et al., 2011). Compared to this study it is believed that the high frequencies observed above are related to population composition, since these studies evaluated animals from veterinary care in poor areas that did not have access to vaccination against leptospirosis, while the majority of the animals in the present study was vaccinated against leptospirosis over six months.

Dogs are accidental hosts of the Icterohaemorrhagiae serogroup, and when infected, develop signs such as high temperature, jaundice and hemorrhage that may lead to death (ELLIS, 2014). The importance of this serogroup as a cause of severe illness in humans should be highlighted. The reservoirs of this serogroup are rodents, especially *Ratus norvergicus,* and the occurrence of the disease is related to the high levels of rainfall and a deficit of adequate sanitary conditions (FAINE et al., 1999).

The serogroup Grippotyphosa has as most adapted hosts wild mammals (HARTSKEERL; TERPSTRA, 1996), however, dogs may get infected with this serogroup (BATISTA et al., 2004; FERNANDES et al., 2013). The municipality of João Pessoa has a natural reserve of Atlantic Forest which extends through 23 boroughs and hosts several species of wild mammals, including rodents and marsupials (PARAÍBA, 2014), which may act as sources of infection for dogs (CORREA, 2007). The serogroup Canicola has already been described in Paraíba with a frequency of 2.1% (BATISTA et al., 2005). Dogs are maintenance hosts of this serogroup, eliminating it through the urine and acting as sources of infection for other animals and humans (ELLIS, 2014). The low frequency of seropositive animals for this serogroup was expected since it has an adaptability for dogs and therefore the antibody titers produced may not have been sufficient to be detected in the serological test.

The serogroup Djasiman was found in dogs in Brazil, in the municipality of Botucatu, with frequencies of 8.7% and 2.3% (COIRO et al., 2011; LANGONI et al., 2013). This serogroup is broadly described in a great variety of wild animals (VIERA et al., 2016; LANGONI et al., 2016), however, it has already been isolated in a case of abortion in a bitch in Argentina (ROSSETTI et al., 2005). It should be noted that commercial vaccines for dogs in Brazil do not contemplate this serogroup, so there is no vaccine protection for the animals. In a serological study carried out in stray dogs in Paraiba it was observed a prevalence of 17% for the serogroup Pomona (BATISTA et al., 2004), whereas in this survey it was 2.2%. Dogs are accidental hosts of this serogroup, being swine the main reservoirs.

The results of the univariable analysis for the risk factors with the most associated variables ($P \leq 0.2$) are presented in Table 2. In the final model of logistic regression the identified risk factors were (Table 3): age from 49 to 72 months (odds ratio = 2.47), age > 72 months (odds ratio = 3.22), and monthly cleaning of the environment where the animals are kept (odds ratio =10.70). The final model presented a good fit (Hosmer and Lemeshow test: chi-square = 0.620; degrees of freedom = 3; $P = 0.892$).

The age groups from 48 to 72 months and > 72 months were identified as risk factors for leptospirosis, and it has been evidenced by other authors (BATISTA et al., 2005; GHNEIM et al., 2007; ZWIJNENBERG et al., 2008; LANGONI et al., 2013), and such a fact may be explained by the greater possibility of exposure of the animals to the agent according to age. It is known that age of up to one year is a protective factor against *Leptospira* sp. infection, and it can be justified by the greater care that the owners have with puppies and the colostral immunity of vaccinated mothers. (OLIVEIRA-LAVINSKY et al., 2014).

The cleaning of the environment only monthly was also a risk factor for leptospirosis. Inadequate sanitary conditions are a classical risk factor for urban and rural leptospirosis. With the accumulation of residues there is the increase of the contact with rodents, which facilitates the emergence of the infections (HAAKE; LEVETT, 2014). It is worth mentioning that the odds

ratio values for this variable (10.70) may not reflect the real risk of infection, since the number of positive animals was small (n = 2), which contributes for the increase of the odds ratio and amplification of the confidence interval of 95%.

Conclusions

It is concluded that dogs attended at veterinary clinics in João Pessoa, Paraíba state, Northeastern Brazil, are exposed to *Leptospira* sp. infection, with predominance of serogroups maintained by wild animals, as well as it is suggested the periodic cleaning of the environment where the animals are kept.

Ethical approval. This experiment was approved and performed under the guidelines of Ethics Committee for Animal Protocol (010.2016) Use of Federal University of Campina Grande

References

ADLER, B.; MOCTEZUMA, A.P. Leptospira and leptospirosis. *Veterinary Microbiology*, Amsterdam, v.27, n.140 (3-4), p.287-296, 2010.

AZEVEDO, S.S.; FERNANDES, A.R.F.; QUEIROGA, I.M.B.N.; ALVES, C.J.; MORAIS, Z.M.; SANTOS, C.S.A.B.; VASCONCELLOS, S.A. Ocorrência e fatores de risco associados à leptospirose em cães atendidos em hospital veterinário no semiárido paraibano. *Brazilian Journal Veterinary Research and Animal Science*, São Paulo, v.48, n.2, p.161-166, *2011*.

BATISTA, C.S.A.; ALVES, C.J.; AZEVEDO, S. S.; VASCONCELLOS, S. A.; MORAIS, Z. M.; CLEMENTINO, I. J.; ALVES, F. A. L.; LIMA, F. S.; ARAUJO NETO, J. O. Soroprevalência e fatores de risco para a leptospirose em cães de Campina Grande, Paraíba. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, 57, n.2 suppl, p.179-185, 2005.

BATISTA, C.S.A.; AZEVEDO, S.S.; ALVES, C.J.; VASCONCELLOS, S.A.; MORAIS, Z.M; CLEMENTINO, I.J.; LIMA, F.S.; ARAÚJO NETO, J.O. Soroprevalência de leptospirose em cães errantes da cidade de Patos, Estado da Paraíba, Brasil. *Brazilian Journal of Veterinary Research and Animal Science*, São Paulo, v. 41, n.2, p.131-136, 2004.

BOURHY, P.; COLLET, L.; BRISSE, S.; PICARDEAU, M. *Leptospira mayottensis* sp. nov., a pathogenic species of the genus *Leptospira* isolated from humans. *International Journal of Systematic and Evolutionary Microbiology*, London, v.64, n.1, p. 4061–4067, 2014.

BROWN, C.A.; ROBERTS, A.W.; MILLER, M.A.; DAVIS, D.A.; BROWN, S.A.; BOLIN, C.A.; JARECKI-BLACK, J.; GREENE, C.E.; MILLER-LIEBL D. Leptospira interrogans serovar Grippotyphosa infection in dogs. Journal of the American Veterinary Medical Association, Schaumburg, v.209, n.7, p.1265-1267, 1996.

CANATTO, B.D.; SILVA, E.A.; BERNARDI, F.; MENDES, M.C.N.C.; PARANHOS, N.T.; DIAS, R.A. Caracterização demográfica das populações de cães e gatos supervisionados do município de São Paulo. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v.64, n.6, p.1515-1523, 2012.

COIRO, C.J.; LANGONI, H.; SILVA, R.C.; ULLMANN, L. S. Fatores de risco para leptospirose, leishmaniose, neosporose e toxoplasmose em cães domiciliados e peridomíciliados em Botucatu-SP. *Veterinária e Zootecnia*, Botucatu, v.18, n.3, p.393-407, 2011.

COLE, J.R.; SULZER, C.R.; PURSELL A.R. Improved microtechnique for the leptospiral microscopic agglutination test. *Applied Microbiology*, Washington, v.25, n.6, p.976–980, 1973.

CORREA S.H.R. Leptospirose. In: CUBAS Z.S. (Org). *Tratado de Animais Silvestres – Medicina Veterinária*. São Paulo: Roca, 2007. p. 736-741.

DELBEM, A.C.B.; FREITAS, J.C.; BRACARENSE, A.P.F.R.L.; MÜLLER, E.E.; OLIVEIRA, R.C. Leptospirosis in slaughtered sows: serological and histopathological investigation. Brazilian Journal of Microbiology, São Paulo, v.33, n.2, p.174-177, 2002.

DOHOO, I.R.; DUCROT, C.; FOURICHON, C.; DONALD, A.; HURNIK, D. An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. *Preventive Veterinary Medicine*, Amsterdam, v.29, n.3, p.221-239, 1997.

ELLIS, W.A. Leptospirosis. In: ZIMMERMAN, J.J. (Org). *Disease of Swine*. Oxford: Wiley-Blackwell, 2012. p.770-778.

ELLIS, W.A. Animal leptospirosis. In: Adler B. (Ed). *Leptospira and Leptospirosis.* New York: Springer, 2014. p.100-125.

FAINE, S.; ADLER, B.; BOLIN, C.; PEROLAT P. *Leptospira and Leptospirosis.* 2nded. Melbourne: MediSci, 1999. 272p.

FERNANDES, A.R.F.; FERNANDES, A.G.; ARAÚJO, V.J.A.; HIGINO, S.S.S.; SILVA, M.L.C.R.; ALVES, C.J.; AZEVEDO, S.S. Soroepidemiologia da leptospirose canina na região metropolitana de Natal, estado do Rio Grande do Norte. *Brazilian Journal of Veterinary Research and Animal Science*, São Paulo, v.50, n.3, p.226-232, 2013.

GALTON, M.M.; SULZER, C.R.; SANTA ROSA, C.A.; FIELDS, M.J. Application of a microtechnique to the agglutination test for leptospiral antibodies. *Applied Microbiology*, Washington, v.13, n.1, p.81-85, 1965.

GHNEIM, G.S.; VIERS, J.H.; CHOMEL, B.B.; KASS, P.H.; DESCOLLONGES, D.A.; JOHNSON, M.L. Use of a case-control study and geographic information systems to determine environmental and demographic risk factors for canine leptospirosis. *Veterinary Research*, London, v.38, n.1, p.37-50, 2007.

GONÇALVES, L.M.F.; MINEIRO, A.L.B.B.; CARVALHO, S.M.; CAMPOS, A.P.; EVANGELISTA, L.S.M.; PINHO, F.; MOREIRA ,E.C.; COSTA, F.A.L. Pesquisa de aglutininas, antígeno de leptospiras e apoptose em rim de suínos naturalmente infectados por *Leptospira* spp. *Pesquisa Veterinária Brasileira*, Seropédica, v.31, n.7. p.561-568, 2011.

GOVERNO DO ESTADO DA PARAÍBA (PARAÍBA, PB). *Estudo para subsidiar a criação de unidade de conservação de proteção integral da mata do buraquinho – Paraíba*. João Pessoa, 2014.

HAAKE, D.A.; LEVETT, P.N. Leptospirosis in humans. In: Adler B. (ed) *Leptospira and Leptospirosis*. New York: Springer, 2014. p. 67-88.

HARTSKEERL, P.A.; TERPSTRA, W.J. Leptospirosis in wild animals. *The Veterinary Quarterly*, v.18, n.3suppl, p.149-150, 1996.

HASHIMOTO, V.Y.; GARCIA, J.L.; SPOHR, K.A.H.; SILVA, F.G.; ALVES, L.A.; FREITAS, J.C. Prevalência de anticorpos contra *Leptospira* spp. em bovinos, caninos, equinos, ovinos e suínos do município de Jaguapitã, estado do Paraná, Brasil. *Arquivos do Instituto Biológico*, São Paulo, v.77, n.3, p.521-524, 2010.

HOSMER, D.W.; LEMESHOW S. *Applied Logistic Regression.* New York: John Wiley & Sons, 2000. 397p.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATISTICA - IBGE. 2010. *Censo demográfico*. Rio de Janeiro: IBGE, 2010. Disponível em: <http://www2.sidra.ibge.gov.br/bda/tabela/protabl.asp?c=202&z=t&o=3&i=P>. Acesso em: 02 dec.2016.

INSTITUTO NACIONAL DE METEOROLOGIA (INMET). *Sistema de Suporte a decisão na agropecuária*. Brasilia. INMET, 2016. Disponível em: <http://sisdagro.inmet.gov.br:8080/sisdagro/app/monitoramento/bhs> Acesso em 02 dec 2016.

LANGONI, H.; KURIBARA, I.Y.; FERREIRA, L.C.A.P.; ULLMANN, L.S.; SÁNCHEZ, G.P.; LUCHEIS, S.B. Anti-leptospirosis agglutinins in Brazilian capybaras (*Hydrochoerus hydrochaeris*). *Journal of Venomous Animals and Toxins including Tropical Diseases*, London, v.22, n. 1, p. 4, 2016.

LANGONI, H.; SILVA, A.V.; SEGISMUNDO, R.; LUCHEIS, S.B.; PAES A.C. Variáveis epidemiológicas e alterações clínicas, hematológicas e urinárias em cães sororreagentes para *Leptospira* spp. *Semina Ciências Agrárias,* Londrina, v.34, n.2, 765-776, 2013.

MARTINS, C.M.; BARROS, C.C.; GALINDO, C.M.; KIKUTI, M.; ULLMANN, L.S.; PAMPUCH, R.S.; HOFFMANN, J.L.; LANGONI, H.; FERREIRA, F.; MOLENTO M.B.; BIONDO A.W. Incidence of canine leptospirosis in the metropolitan area of Curitiba, State of Paraná, Southern Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, Uberaba, v.46, n.6, p.772-775, 2013.

MIOTTO, B.A.; MORENO, L.Z.; GUILLOUX, A.G.; SOUSA, G.O.; LOUREIRO, A.P.; MORENO, A.M.; LILENBAUM, W.; VASCONCELLOS, S.A.; HEINEMANN, M.B.; HAGIWARA M.K. Molecular and serological characterization of the first *Leptospira santarosai* strain isolated from a dog. *Acta Tropica*, Basel, v.162, n.1, p.1-4, 2016.

MINISTÉRIO DA SAÚDE (BRASIL); Sistema de Informação de Agravos De Notificação – SINAN. Disponível em: < http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sinannet/cnv/leptopb.def> [Acesso em: 03 set 2017]

MIRAGLIA, F.; MORENO, A.M.; GOMES, C.R.; PAIXÃO, R.; LIUSON, E.; MORAIS, Z.M.; MAIORKA, P.C.; SEIXAS, F.K.; DELLAGOSTIN, O.A.; VASCONCELLOS, S.A. Isolation

and characterization of *Leptospira interrogans* form pigs slaughtered in São Paulo State, Brazil. *Brazilian Journal Microbiology*, São Paulo, v.39, n.3, p.501-507, 2008.

OLIVEIRA-LAVINSKY, M.; ABOU R.; REUSS G.; LANGONI H. Seroprevalence of anti-*Leptospira spp* antibodies in Bahia, Brazil. *Preventive Veterinary Medicine*, Amsterdam, v.106, n.1, p.79–84, 2012.

PEREIRA, M. D. B. *As chuvas na cidade de João Pessoa: uma abordagem genética*. 2014. Monografia (Trabalho de conclusão de curso em Curso de Geografia) - Universidade Federal da Paraíba, João Pessoa.

ROSSETTI, C.A.; LIEM, M.; SAMARTINO, L.E.; HARTSKEERL R.A. Buenos Aires, a new *Leptospira* serovar of serogroup Djasiman, isolated from an aborted dog fetus in Argentina. *Veterinary Microbiology*, Amsterdam, v.107, n.3-4, 241-248, 2005.

SHIMABUKURO, F.H.; DOMINGUES, F.H.; LANGONI, H.; SILVA, A.V.; PINHEIRO, J.P.; PADOVANI, C.R. Pesquisa de suínos portadores renais de leptospiras pelo isolamento microbiano e reação em cadeia pela polimerase em amostras de rins de animais sorologicamente positivos e negativos para leptospirose. *Brazilian Journal of Veterinary Research and Animal Science*, São Paulo, v.40, n.4, p.243-253, 2003.

SILVA, F. J. *Epidemiologia da infecção por Leptospira spp. em áreas rurais nos biomas brasileiros*. 2014. Tese (Doutorado em Medicina Veterinária) - Universidade Estadual Paulista, Jaboticabal.

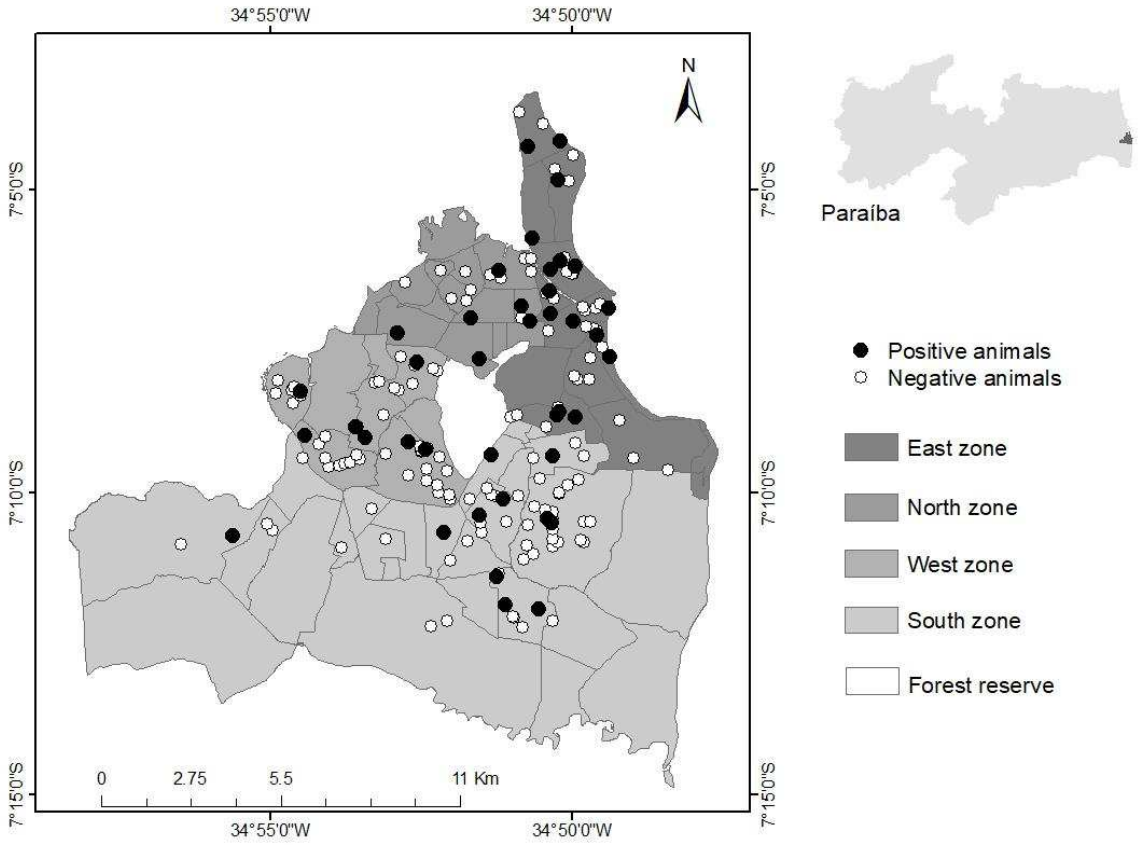
THRUSFIELD, M.. *Epidemiologia Veterinária*. 2.ed. São Paulo: Roca, 2004. 556p.

VIEIRA, A.S.; NARDUCHE, L.; MARTINS, G.; SCHABIB PÉRES, I.A.; ZIMMERMANN, N.P.; JULIANO, R.S.; PELLEGRIN, A.O.; LILENBAUM W. Detection of wild animals as carriers of *Leptospira* by PCR in the Pantanal biome, Brazil. *Acta Tropica*, Basel, v.163, n.87-89, 2016.

WORLD ORGANIZATION FOR ANIMAL HEALTH (OIE). *Reference Laboratory Reports Activities*. United Kingdom OIE 2014. Disponível em: < http://www.oie.int/fileadmin/Home/fr/Our_scientific_expertise/reflabreports/2014/report_204_2014_Leptospirosis_UNITED_KINGDOM.pdf> [Acesso em 02 ago 2016].

ZWIJNENBERG, R.J.; SMYTHE, L.D.; SYMONDS, M.I.; DOHNT, M.F.; TORIBIO J.A. Cross-sectional study of canine leptospirosis in animal shelter populations in mainland Australia. *Australian Veterinary Journal*, Oxford, v.86, n.8, p.317-323, 2008.

Figure 1. Map with the division of the city of João Pessoa in zones and geographical location of the animals, according to positive and negative conditions for infection by *Leptospira* sp. Detail shows the location of João Pessoa within Paraíba.



Source: Elaborated by the authors

Table 1. *Leptospira* sp. seropositivity in dogs attended at veterinary clinics in the municipality of João Pessoa, Paraíba, from April 2015 to May 2016 according to serogroup and respective antibody titers

Serogroup	Antibody titre						Total (%)
	100	200	400	800	1600	3200	
Icterohaemorrhagiae	10	12	5	1	0	0	28 (62.3)
Grippotyphosa	1	6	2	0	0	1	10 (22.2)
Canicola	5	0	0	0	0	0	5 (13.3)
Djasmani	1	0	0	0	0	0	1 (2.2)
Pomona	1	0	0	0	0	0	1(2.2)
Total	18	18	7	1	0	1	45 (100)

Source: Elaborated by the authors

Table 2. Univariable analysis of the risk factors associated with *Leptospira* sp. infection in dogs attended at veterinary clinics in João Pessoa, Paraíba, from April 2015 to May 2016, with variables presenting $P \leq 0.2$.

Variables	Categories	Total no. of animals	No. of seropositive animals (%)	*P*
Age	Up to 48 months	219	16 (7.3)	
	49 to 72 months	63	9 (14.3)	
	> 72 months	102	20 (19.6)	0.005
Access to treated water	No	7	2(28.6)	
	Yes	377	43(11.4)	0.193
Contact with bovine	No	375	42 (11.2)	
	Yes	9	3(33.3)	0.076
Contact with equine	No	375	42(11.2)	
	Yes	9	3(33.3)	0.076
Contact with wild	No	368	41 (11.1)	
Animals	Yes	16	4 (25)	0.104
Contact with goats	No	377	42(11.1)	
	Yes	7	3 (42.9)	0.038
Contact with sheep	No	376	42 (11.2)	
	Yes	8	3 (37.5)	0.055
Contact with swine	No	377	42 (11.1)	
	Yes	7	3 (42.9)	0.038
Cleaning and disinfection of the environment where the animals are kept	Daily	287	36 (12.5)	
	Weekly/fortnightly	93	7 (7.5)	
	Monthly	4	2 (50)	0.024
Vaccination	No	46	1(2.2)	
	Yes	338	44 (13)	0.028
Presence of ticks	No	174	27(15.5)	
	Yes	210	18(8.6)	0.039

Source: Elaborated by the authors

Table 3. Risk factors associated with leptospirosis in dogs attended at veterinary clinics of João Pessoa, Paraíba, from April 2015 to May 2016, estimated by multiple logistic regression.

Risk factors	Regression coefficient	Standard error	Wald	Degrees of freedom	Odds ratio	95% CI	*P*
Age from 49 to 72 months	0.906	0.456	3.941	1	2.47	[1.012 -6.053]	0.047
Age > 72 months	1.170	0.373	9.850	1	3.22	[1.552 – 6.688]	0.002
Monthly cleaning of the environment where the animals are kept	2.376	1.114	4.552	1	10.70	[1.213- 95.535]	0.033

Hosmer and Lemeshow test: chi-square = 0.620; degrees of freedom = 3; $P = 0.892$

Source: Elaborated by the authors

4. CAPITULO II

PREVALENCE AND RISK FACTORS ASSOCIATED WITH *Toxoplasma gondii* AND *Neospora caninum* INFECTION IN DOGS ATTENDED AT VETERINARY CLINICS IN JOÃO PESSOA, PARAÍBA.

"Prevalência e fatores de risco associados à infecção por *Toxoplasma gondii* e *Neospora caninum* em cães atendidos em clínicas veterinárias de João Pessoa, Paraíba."

(**Artigo submetido à revista Revista Brasileira de Parasitologia Veterinária, Qualis A2**)

Prevalence and risk factors associated with *Toxoplasma gondii* and *Neospora caninum* infections in dogs attended at veterinary clinics in João Pessoa, Paraíba state, Northeastern Brazil

Prevalência e fatores de risco associados às infecções por *Toxoplasma gondii* e *Neospora caninum* em cães atendidos em clínicas veterinárias de João Pessoa, Estado da Paraíba, Nordeste do Brasil

Arthur Willian de Lima Brasil[1], Roberta Nunes Parentoni[1], José Givanildo da Silva[2], Carolina de Sousa Américo Batista Santos[1], Rinaldo Aparecido Mota[2], Sérgio Santos de Azevedo[1*].

[1]Laboratório de Doenças Transmissíveis, Programa de Pós-graduação em Medicina Veterinária, Universidade Federal de Campina Grande – UFCG, Patos, PB, Brasil.
[2]Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco – UFRPE, Recife, PE, Brasil.

Abstract

The aim of this study was to determine the prevalences of infections due to *Toxoplasma gondii* and *Neospora caninum* and corresponding risk factors among dogs attended at veterinary clinics in the city of João Pessoa, Paraíba, northeastern Brazil. Blood samples were collected from 384 dogs that were attended at 34 veterinary clinics between April 2015 and May 2016. These two agents were diagnosed through the indirect immunofluorescence reaction (IFAT). Among the 384 animals evaluated, 37 (9.6%) were positive for *T. gondii*, with titers ranging from 16 to 512. Six dogs (1.6%) were positive for *N. caninum*, with titers of 50 to 200. Access to the streets (OR = 4.60; 95% CI = 1.74-12.20) and environments close to forested areas (OR = 2.79; 95% CI = 1.32-5.93) were found to be risk factors for *T. gondii* infection. It is recommended that dogs in this region do not transit close to forested areas and it is suggested that animals that go onto the streets should not have access to contaminated water, food and environments.

Key words: Toxoplasmosis, Neosporosis, Domiciled dogs, Northeastern Brazil

Resumo

O objetivo deste trabalho foi determinar a prevalência e os fatores de risco das infecções por *Toxoplasma gondii* e *Neospora caninum* em cães atendidos em clínicas veterinárias da cidade de João Pessoa, Paraíba, Nordeste do Brasil. Foram colhidas 384 amostras de sangue cães de procedentes de atendimento 34 clínicas veterinárias no período de abril de 2015 a maio de 2016. O diagnóstico para os dois agentes foi realizado pela reação de imunofluorescência indireta (RIFI). Dos 384 animais avaliados 37 (9,6%) foram positivos para *T. gondii* com títulos variando de 16 a 512. Para *N. caninum* seis (1,6%) cães foram positivos com títulos de 50 a 200. Foram constatados como fatores de risco para *T. gondii* as variáveis acesso à rua (OR = 4,60; IC 95% = 1,74-12,20) e ambiente próximo a matas (OR= 2,79; IC 95% = 1,32-5,93). Recomenda-se que os cães dessa região não transitarem próximos a matas e sugere-se que os animais que frequentam a rua não tenham acesso à água, alimentos e ambiente contaminados.

Palavras-chave: Toxoplasmose, Neosporose, Cães domiciliados, Nordeste do Brasil

Introduction

The interrelations among animals, humans and the environment have intensified over recent decades, which has been causing a variety of ecological imbalances. From a public health point of view, these changes have taken on great importance, given that there is a real possibility of increased transmission of zoonoses (ZINSSTAG et al., 2011). Thus, pets such as dogs, which are increasingly incorporated into family groups as members of the family, may play a major role in maintaining and transmitting infectious agents. They may also act as sentinels for their respective diseases (ULLMAN et al., 2008). For this reason, monitoring of diseases like toxoplasmosis and neosporosis is needed and their risks towards the ecosystem need to be assessed.

Toxoplasmosis and neosporosis are caused by *Toxoplasma gondii* and *Neospora caninum*, respectively, which are obligate intracellular coccidian protozoa belonging to the phylum Apicomplexa. Cats and dogs are the definitive hosts of *T. gondii* and *N. caninum*, respectively. Transmission of these parasites takes place through ingestion of oocysts that are eliminated from the feces of the definitive hosts; through ingestion of tissue cysts that are present in the musculature of infected animals; and through the

transplacental route (DUBEY, 2010; DUBEY, 2003). *T. gondii* is considered to be a zoonotic agent (DUBEY, 2010). On the other hand, there is no evidence of zoonotic characteristics regarding *N. caninum*, although some studies have demonstrated that humans can present antibodies against this protozoon (OSHIRO et al., 2015). Even though dogs are not definitive hosts for *T. gondii*, they have an important role in mechanical dissemination of the agent (FRENKEL et al., 2003).

In Brazil, recent studies on the seroprevalence of *T. gondii* in dogs demonstrated variation from 11.5% to 70.8% (DANTAS et al., 2013; LANGONI et al., 2013), while for *N. caninum* this variation was from 4.2% to 43.1% (SOUSA et al., 2012; RAIMUNDO et al., 2015). The main risk factors reported in relation to *T. gondii* were the following: contact with cats (RODRIGUES et al., 2016); male sex (LANGONI et al., 2013; RODRIGUES et al., 2016); and greater age and access to the streets (AZEVEDO et al., 2005; DANTAS et al., 2013; RAIMUNDO et al., 2015). In relation to *N. caninum*, the risk factors were homemade diets, rural environment, greater age, cleansing of the environment only done weekly and presence of rats (BRESCIANI et al., 2007, PARADIES et. al., 2007, DANTAS et al., 2013).

Epidemiological surveys on toxoplasmosis and neosporosis among dogs are of strategic public health importance, because they provide an overview of the dynamics of these diseases in a given region. Such knowledge is of major importance in places like the city of João Pessoa, Paraíba, northeastern Brazil, which has a climate and environment that are favorable for parasite development. In this light, the aim of this study was to ascertain the seroprevalence of *T. gondii* and *N. caninum* and the risk factors associated with infections in dogs attended at veterinary clinics in João Pessoa.

Material e Methods

Study area

The survey was conducted in the municipality of João Pessoa, capital city of the state of Paraíba, which has about 720 thousand inhabitants (BRASIL, 2010), distributed in 59 boroughs, and with an area of preserved Atlantic Forest in the center of the urban spot and bordered by mangroves (PARAÍBA, 2014). Its climate is humid tropical, with annual average temperatures of 23ºC and annual rainfall rate above 1,906 mm (PEREIRA, 2014).

Sampling

The selection of the veterinary clinics was based on data from the Regional Council of Veterinary Medicine (CRMV-PB), which has informed the existence of 40 registered clinics, however, in the on-the-spot verification four establishments had closed and in two others the owners preferred not to participate. Then, 34 veterinary clinics in the municipality of João Pessoa distributed in the four zones of the city (north, south, east and west) were used.

The minimum number of animals was determined by the formula for simple random sampling (THRUSFIELD, 2004):

$$n = \frac{Z^2 \text{ x } P\ (1 - P)}{d^2}$$

Where:

n = number of sampled animals

Z = value of the normal distribution for the confidence level of 95%

P = expected prevalence of 50% (sampling maximization)

d = error of 5%

In total, 384 apparently healthy dogs, unvaccinated or vaccinated over six months against leptospirosis were selected. Females in the periparturient and lactation periods were excluded from the study. The collections were carried out in the period from April 2015 to May 2016. Samples were collected by venipuncture of the cephalic or jugular vein, using vacuum tubes of 5 mL, and sera were placed into microtubes and frozen until the serologic tests were performed.

Serological diagnosis of infections due to T. gondii and N. caninum

To detect anti-*T. gondii* and anti-*N. caninum* antibodies, the indirect immunofluorescence reaction (IFAT) was used. For *T. gondii*, the dilution of 1:16 was taken to be the cutoff point (LANGONI et al., 2013) and the technique was performed in accordance with the methodology described by Camargo (1974), using tachyzoites from the RH strain as the antigen. For *N. caninum*, the cutoff point was the dilution of 1:50 (ACOSTA et al., 2016) and the technique was performed in accordance with Paré et al. (1995), using the NC-1 strain from *N. caninum* cultured in bovine monocytes as

the antigen. In both tests, both positive and negative serum samples were used as controls.

Risk factor analysis

Epidemiological questionnaires were applied to dog owners in order to obtain data to be used in the risk factor analysis. The analyzed variables and their respective categories were: educational level of the owner (illiterate, incomplete primary school, complete primary school, incomplete high school, complete high school, incomplete university degree, university degree), gender (female, male), breed (pure-bred, crossbred), age (up to 48 months, 49 to 72 months, above 72 months), access to street (no, yes), feed (commercial food, homemade food, food scraps, raw meat), access to treated water (no, yes), reason for keeping the dog (company, guard, other), contact with dogs (no, yes), contact with bovine (no, yes), contact with equine (no, yes), contact with wild animals (no, yes), contact with cats (no, yes), contact with goats (no, yes), contact with sheep (no, yes), contact with swine (no, yes), type of housing (masonry, shacks, stilts, mud huts), where the animal is kept (soil, cement, soil/cement), environment where the animal lives (rural or urban), environment near woodlands area (no, yes), regular garbage collection (no, yes), cleaning and disinfection of the environment (daily, weekly/fortnightly, monthly), vaccination (no, yes), contact with flooded areas (no, yes), presence of rodents (no, yes), occurrence of abortions (no, yes), occurrence of stillbirths (no, yes), presence of ticks (no, yes).

An univariable exploratory analysis of the data was carried out for the selection of variables with $P \leq 0.2$ using the chi-square or Fischer exact tests. Subsequently the significant variables passed to a multivariable analysis using the multiple logistic regression with significant level of 5% (HOSMER; LEMESHOW, 2000). The adjustment of the final model was verified with the Hosmer and Lemeshow test, by which a value of $P \geq 0.05$ indicates a good fit. The collinearity between independent variables was verified by correlation analysis; for the variables with strong collinearity (correlation coefficient > 0.9), one of the two variables was excluded from the multiple analysis according to the biological plausibility (DOHOO et al., 1997). Confounding was evaluated by the monitoring the alterations in the model's parameters (> 20%) when adding new variables. Data analyses were carried out using the SPSS 23.0 *for* Windows software.

Results

Among the 384 animals analyzed, 37 (9.6%) were seropositive for *T. gondii*, with the following respective antibody titers and frequencies: 16 (35.1%), 32 (18.9%), 64 (8.1%), 128 (13.5%), 256 (16.3%) and 512 (8.1%). For *N. caninum*, six dogs (1.6%) were seropositive, with titers and frequencies of 50 (66.8%), 100 (16.6%) and 200 (16.6%), respectively. One animal was seropositive for both parasites. Figure 1 presents the geographical locations of the homes of the dogs that were positive and negative for *T. gondii* (Fig. 1A) and *N. caninum* (Fig. 1B) in João Pessoa.

In univariable analysis on the risk factors associated with *T. gondii* (Table 1), the following variables were selected: type of rearing, access to treated water, aim of rearing, contact with dogs, contact with wild animals, environment in which the animal lived, locality, frequency of cleansing of the environment and contact with rats. Through multivariable analysis (Table 2), the variables of access to the streets (OR = 4,60; 95% CI = 1.74-12.20) and environment close to forested areas (OR = 2.79; 95% CI = 1.32-5.93) were identified as risk factors for *T. gondii*. There were no risk factors for *N. caninum*.

Discussion

The results found in the present study regarding the prevalence of *T. gondii* are close to those found by Dantas et al. (2013) in Natal, Rio Grande do Norte, who observed that 11.5% of the dogs were seropositive; and by Dantas et al. (2014) in Patos, Paraíba, who observed that 15.6% were seropositive. Both of those populations consisted of dogs attended at veterinary clinics.

However, in an evaluation on dogs during a vaccination campaign in Campina Grande, Paraíba, Azevedo et al. (2005) found a prevalence of 45.1%. In this context, since the serological diagnostic method used was the same in these studies, it is believed that the characteristics of the canine population may have influenced the seroprevalence of toxoplasmosis. The dogs of the present study were attended at veterinary clinics, which leads us to believe that they were receiving better care from their owners regarding hygiene and health than were dogs that were vaccinated through public campaigns.

Although dogs that are regularly attended at veterinary clinics have received better care regarding hygiene and health (DANTAS et al., 2013), these animals may nonetheless acquire oocysts of *T. gondii* from the environment or through contact with the feces of infected cats. These oocysts may adhere to the animals' fur, which gives rise to the possibility that humans might become infected through contact with these animals. One important point is that dogs may act as sentinels for toxoplasmosis. Thus, epidemiological monitoring of the behavior of this zoonosis in these animals is immensely important (NAVA, 2008).

Dogs play a fundamental role in transmission of neosporosis to other animals, especially to cattle. Neosporosis in cattle causes economic losses relating to abortions of the order of US$ 1.2 billion worldwide (REICHEL et al., 2013). High prevalences of anti-*N. caninum* antibodies in dogs are generally correlated with living in rural areas (ROBBE et al., 2016), which may explain the low frequency of seropositive dogs in the present study, given that all the dogs investigated originated from the urban zone of João Pessoa.

Access to the streets among these dogs was a risk factor for infection with *T. gondii* in the present study. Similar results were described by Lopes et al. (2011) in Portugal, such that dogs with access to the streets had a higher chance of presenting anti-*T. gondii* antibodies. Dantas et al. (2013) stated that dogs that had access to the streets were 4.6 times more likely to be seropositive for *T. gondii*. Access to the streets provides greater possibilities for dogs to eat intermediate hosts such as birds and rodents, which are common sources of *T. gondii* infection (SVOBODA & SVOBODOVÁ, 1987), and for them to consume sporulated oocysts that are present in water and in the environment.

Gennari et al. (2015) evaluated wild rodents and marsupials of the Atlantic Forest in the state of São Paulo and found that some individuals were seropositive for *T. gondii*. This suggested that these animals might be sources of infection for the fauna of the region and for domestic animals. In the present study, it was observed that dogs living in homes that were close to forested areas presented greater risk of infection with *T. gondii*. In the municipality of João Pessoa, there is an area of preserved Atlantic Forest (Figure 1) covering an area of more than 515 hectares that borders 25 districts of the city (CAVALCANTI, 2013). Moreover, there are several other areas with thick plant cover. Because of this proximity between city districts and forested areas, it is

believed that this facilitates contact with a wide variety of sources of *T. gondii* infection, which increases the possibility of disease transmission.

The prevalence of toxoplasmosis and the risk factors for this disease found among the dogs of this study emphasize the importance of monitoring not only this disease in the canine population, but also various other zoonotic diseases of importance for public health. The changes that have stemmed from urbanization and deforestation of previously unexploited areas have altered the behavior and speed of propagation and appearance of these diseases. Thus, an in-depth approach addressing the ecosystem, based on multidisciplinary concepts of public health, is needed (KARESH et al., 2012; MWANGI et al., 2016).

Conclusion

The dogs attended at veterinary clinics in João Pessoa are exposed to infections caused by *T. gondii* and *N. caninum*. This is a matter of concern from a public health point of view, especially in relation to toxoplasmosis, which is an important zoonosis with involvement of environmental factors. Based on the risk factors, it can be suggested that greater care should be taken regarding dogs that have access to the streets and that do not transit in environments close to forested areas.

References

Acosta ICL, Centoducatte LD, Soares HS, Marcili A, Gondim MFN, Rossi Junior JL, et al. Occurrence of *Neospora caninum* and *Toxoplasma gondii* antibodies in dogs from rural properties surrounding a biological reserve, Espirito Santo, Brasil. *Rev. Bras. Parasitol. Vet* 2016; 25 (4): 536-539.

Azevedo SS, Batista CS, Vasconcellos SA, Aguiar DM, Ragozo AM, Rodrigues AA, et al. Seroepidemiology of *Toxoplasma gondii* and *Neospora caninum* in dogs from the state of Paraíba, Northeast region of Brazil. *Res. Vet. Sci.* 2005; 79(1):51-56.

Bresciani KDS, Costa AJ, Navarro IT, Toniollo GH, Sakamoto CAM, Arantes TP et al. Toxoplasmose canina: aspectos clínicos e patológicos *Semin Cien Agrar*, 2008; 29(1): 189-202.

Camargo ME. Introdução das técnicas de imunofluorescência. *Rev Bras Patol Clin* 1974; 10(3): 87-107.

Cavalcanti RG. *Influência da mata do buraquinho sobre a qualidade da água do rio jaguaribe* [Monografia]. João Pessoa: Universidade Federal da Paraíba; 2013.

Dantas SBA, Fernandes ARF, Souza Neto OL, Mota RA, Alves CJ, Azevedo SS. Ocorrência e fatores de risco associados às infecções por *Toxoplasma gondii* e *Neospora caninum* em cães no município de Natal, Estado do Rio Grande do Norte, Nordeste do Brasil. *Cienc. Rural* 2013; 43(11): 2042-2048.

Dantas SBA, Fernandes ARF, Souza Neto OL, Mota RA, Alves CJ, Azevedo SS. Fatores de risco para a ocorrência de anticorpos contra *Toxoplasma gondii* e *Neospora caninum* em cães domiciliados no Nordeste do Brasil. *Semin Cien Agrar*, 2014; 35(2):875-882.

Dohoo IR, Ducrot C, Fourichon C, Donald A, Hurnik D. An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. *Prev Vet Med* 1997; 29(3): 221-239.

Dubey JP. Review of *Neospora caninum* and neosporosis in animals. *Korean J Parasitol* 2003; 41(1):1-16.

Dubey JP. *Toxoplasmosis of Animals and Humans*. New York: CRC Press Taylor & Francis Group; 2010.

Frenkel JK, Lindsay DS, Parker BB, Dobesh M. Dogs as possible mechanical carriers of Toxoplasma, and their fur as a source of infection of young children. *Int J Infect Dis*, 2003; 7(1):292-293.

Gennari SM, Ogrzewalska MH, Soares HS, Saraiva DG, Pinter A, Nieri-Bastos FA, et al. *Toxoplasma gondii* antibodies in wild rodents and marsupials from the Atlantic Forest, state of São Paulo, *Brazil. Braz. J. Vet. Parasitol* 2015; 24(3):379-382.

Governo do Estado da Paraíba**.** *Estudo para subsidiar a criação de unidade de conservação de proteção integral da mata do buraquinho – Paraíba* [online]. 2014 [cited 2017 Jun 26]. Available from: http://sol.sudema.pb.gov.br/arquivos/sol/download/propostacriacaomataburaquinho.pdf.

Hosmer DW, Lemeshow S. *Applied logistic regression*. 2nd ed. New York: John Wiley & Sons; 2000.

Karesh WB, Dobson A, Lloyd-Smith JO, Lubroth J, Dixon MA, Bennett M, Aldrich S, et al. Ecology of zoonoses: natural and unnatural histories. *Lancet* 2012; 380 (9857): 1936-1945.

Langoni H, Fornazari F, Silva RC, Monti ET, Villa FB. Prevalence of antibodies against *Toxoplasma gondii* and *Neospora caninum* in dogs. *Braz J Microbiol* 2013; 44(4):1327-1330.

Lindsay DS, Dubey JP, Butler JM, Blagburn BL. Mechanical transmission of *Toxoplasma gondii* oocysts by dogs. *Vet Parasitol* 1997; 73(1): 27-33.

Lopes AP, Santos H, Neto F, Rodrigues M, Kwok OCH, Dubey JP et al. Prevalence of Antibodies to *Toxoplasma gondii* in Dogs From Northeastern Portugal. *J Parasitol* 2011; 97(3): 418-420.

Mwangi W, Figueiredo P, Criscitiello MF. One Health: Addressing Global Challenges at the Nexus of Human, Animal, and Environmental. *Health PLoS Pathog* 2016; 12(9): 1-8.

Nava, AFD. *Espécies sentinelas para a Mata Atlântica: as conseqüências epidemiológicas da fragmentação florestal no Pontal do Paranapanema, São Paulo*. [Tese]. São Paulo: Universidade de São Paulo; 2008.

Oshiro LM, Motta-Castro ARC, Freitas SZ, Cunha RC, Dittrich RL, Meirelles ACF, et al. *Neospora caninum* and *Toxoplasma gondii* serodiagnosis in human immunodeficiency virus carriers. *Rev Soc Bras Med Trop* 2015; 48(5):568-572.

Paradies P, Capelli G, Testini G, Cantacessi C, Trees AJ, Otranto D. Risk factors for canine neosporosis in farm and kennel dogs in southern Italy. *Vet Parasitol*. 2007 30(145) 3-4: 240-244.

Paré J, Hietala SK, Thurmond MC. An enzyme-linked immunosorbent assay (ELISA) for serological diagnosis of *Neospora* sp. infection in cattle. *J Vet Diagn Invest* 1995; 7(3): 352-359.

Pereira, M.D.B. As chuvas na cidade de João Pessoa: uma abordagem genética. [Monografia]. João Pessoa: Universidade Federal da Paraíba; 2014.

Raimundo JM, Guimarães A, Moraes LMB, Santos LA, Nepomuceno LL, Barbosa SM, et al. *Toxoplasma gondii* and *Neospora caninum* in dogs from the state of Tocantins: serology and associated factors. *Rev Bras Parasitol Vet* 2015; 24(4): 475-481.

Reichel MP, Ayanegui-Alcérreca MA, Gondim LF, Ellis JT. What is the global economic impact of *Neospora caninum* in cattle - the billion dollar question. *Int J Parasitol*. 2013; 43(2):133-142.

Robbe D, Passarelli A, Gloria A, Di Cesare A, Capelli G, Iorio R, et al. *Neospora Caninum* Seropositivity and Reproductive Risk Factors in Dogs. *Exp Parasitol* 2016; 164: 31-35.

Rodrigues JY, Almeida ABPF, Boa Sorte EC, Gasparetto ND, Cruz FACS, Sousa VRF. Seroprevalence of *Toxoplasma gondii* in dogs of riverside communities of Mato Grosso Pantanal, Brazil. *Rev Bras Parasitol Vet* 2016; 25(4): 531-535.

Svoboda M, Svobodová V. Effects of breed, sex, age, management and nutrition on the incidence of *Toxoplasma gondii* antibodies in dogs and cats. *Acta Veterinária Brno* 1987; 56(1): 315 - 330.

Sousa ME, Porto WJN, Albuquerque PPF, Neto OLS, Júnior JWP Mota R.A. Seroprevalence of antibodies to *Neospora caninum* in dogs in the state of Alagoas, Brazil. *Rev Bras Parasitol Vet* 2012; 21(3): 287-290.

Thrusfield, M. *Epidemiologia Veterinária*. 2.ed. São Paulo: Roca; 2004.

Ullmann LS, Guimarães FF, Fornazari F, Tomé, RO, Camossi LG, Greca H, et al. Ações de vigilância continuada, papel do cão como animal sentinela para toxoplasmose. *Rev Bras Parasitol Vet* 2008;17(1): 345-347.

Zinsstag J, Schelling E, Waltner-Toews D, Tanner M. From "one medicine" to "one health" and systemic approaches to health and well-being. *Prev Vet Med* 2011;101(3-4):148-156.

Table 1. Univariate analysis (P ≤ 0.20) of the risk factors associated with seropositivity for *T. gondii* in dogs attended at veterinary clinics in João Pessoa-PB, from April 2015 to May 2016.

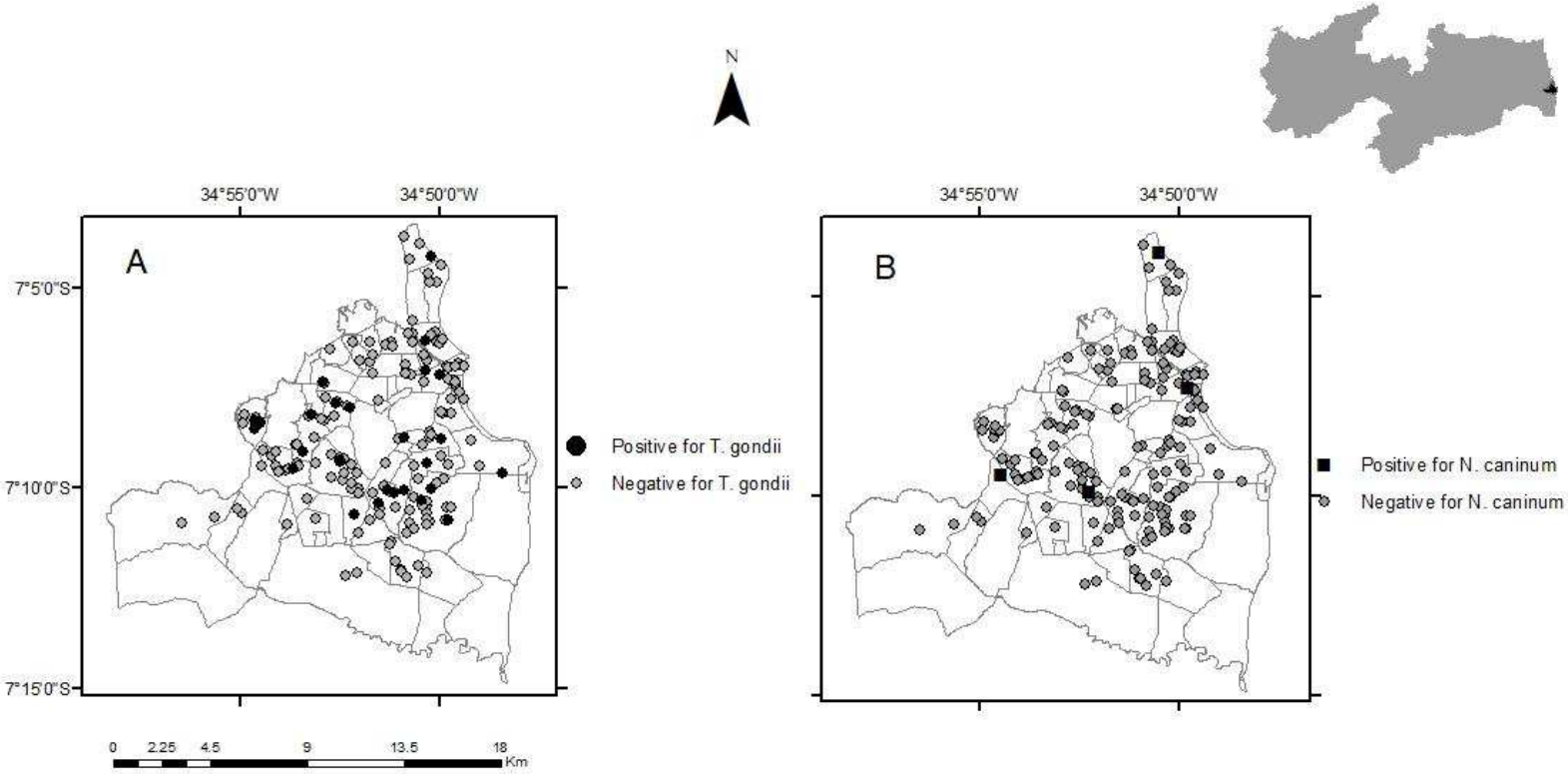
Variables	Categories	Total nºof animals	Nº seropositives animals (%)	*P*
Access to street	No	358	30 (8.4)	0.002
	Yes	26	7 (26.9)	
Access to treated water	Não	7	2 (28.6)	0.139
	Sim	377	5 (9.3)	
Reason for keeping the dog	Company	269	21 (7.8)	0.115
	Guard	68	8 (11.8)	
	Other	47	8 (17.0)	
Contact with dogs	No	68	10 (14.7)	0.171
	Yes	316	27 (8.5)	
Contact with wild animals	No	368	33 (9.0)	0.057
	Yes	16	4 (25.0)	
Where the animal is kept	Cement	243	19 (7.8)	0.032
	Soil/Cement	116	12 (10.3)	
	Soil	25	6 (24.0)	
Environment where the animal lives	Rural	30	5 (16.7)	0.191
	Urban	354	32 (9.0)	
Environment near woodlands areas	No	192	11 (5.7)	0.009
	Yes	192	26 (13.5)	
Cleaning and disinfection of the environment	Daily	287	23 (8.0)	0.138
	Weekly/ fortnightly	93	13 (14.0)	
	Monthly	4	1 (25.0)	
Presence of rodents	No	206	15 (7.3)	0.093
	Yes	178	22 (12.4)	

Table 2. Risk factors associated with seropositivity for *T. gondii* in dogs attended at veterinary clinics in João Pessoa-PB, from April 2015 to May 2016

Varible	Logistic regression coefficient	Standard error	Wald	Degrees of freedom	*Odds Ratio*	CI 95%	*P*
Access to street	1.527	0.497	9.421	1	4.60	[1.74-12.20]	0.002
Environment near woodlands areas	1.028	0.384	7.166	1	2.79	[1.32-5.93]	0.007

Hosmer e Lemeshow test: Chi-square = 0,102; Degrees of freedom = 1; *P* = 0,749

Figure 1. Geographic location of dogs attended at veterinary clinics in the city of Joao Pessoa-PB, from April 2015 to May 2016, according to the serological condition for *T. gondii* (Fig. 1A) and *N. caninum* (Fig. 1B). The detail shows the city of João Pessoa in the state of Paraíba.



5. CAPITULO III

PREVALENCE ATND RISK FACTORS ASSOCIATED WITH *Leishmania* spp. AND *Trypanosoma cruzi* INFECTIONS IN DOGS ATTENDED AT VETERINARY CLINICS IN THE CITY OF JOÃO PESSOA, PARAÍBA STATE, NORTHEASTERN BRAZIL

"Prevalência e fatores de risco associados às infecções por *Leishmania* spp. e *Trypanosoma cruzi* em cães atendidos em clínicas veterinárias da cidade de João Pessoa, Estado da Paraíba, Nordeste do Brasil"

(Artigo submetido à revista Semina: Ciências Agrárias, Qualis B1)

Prevalence atnd risk factors associated with *Leishmania* spp. and *Trypanosoma cruzi* infections in dogs attended at veterinary clinics in the city of João Pessoa, Paraíba state, Northeastern Brazil

Prevalência e fatores de risco associados às infecções por *Leishmania* spp. e *Trypanosoma cruzi* em cães atendidos em clínicas veterinárias da cidade de João Pessoa, Estado da Paraíba, Nordeste do Brasil

Arthur Willian de Lima Brasil[1]; Dália Monique Ribeiro Machado[2]; Mariana Aimee Ramos Xavier da Silva[2]; Noelle de Castro Barrosa[2]; Raizza Barros Sousa Silva[1]; Márcia Almeida de Melo[1]; Hélio Langoni[2]; Sérgio Santos de Azevedo[1*].

[1]Centro de Saúde e Tecnologia Rural - Universidade Federal de Campina Grande (UFCG), Patos, PB, Brasil.

[2] Departamento de Higiene Veterinária e Saúde Pública, da FMVZ/UNESP - Campus de Botucatu (SP).

CORRESPONDÊNCIA: S.S. Azevedo [sergio@vps.fmvz.usp.br – Tel.: +55 (83) 99951-0999]. Laboratório de Doenças Transmissíveis s, Centro de Saúde e Tecnologia Rural - Universidade Federal de Campina Grande (UFCG). Av. Universitária, s/n, Santa Cecília, CEP 58700- 970 Patos, PB, Brasil.

Abstract

The aim of this work was to determine the prevalence and the risk factors associated with the infection by *Leishmania* spp. and *Trypanosoma cruzi* in dogs attended at veterinary clinics in the municipality of João Pessoa, Paraíba state, Northeastern Brazil. A total of 384 blood samples collected from dogs from 34 veterinary clinics during April 2015 to May 2016 were used. *Leishmania* spp. serological diagnosis was performed by the indirect fluorescent antibody test (IFAT) using *Leishmania major* promastigotes as antigens and cut-off point of 1:40. The reagent sera were submitted to a confirmatory test using the ELISA S7. The animals were considered positive when the samples reacted to both tests. For the serological diagnosis of *T. cruzi* the IFAT was also carried out, with *T. cruzi* epimastigotes (Y strain) as antigens and cut-off point of 1:40. The seroprevalence of *Leishmania* spp. was 2.8% (11/384), and for *T. cruzi* it was 1.5% (6/384). Two animals were found to be positive for both parasites. The variable access to street was identified as risk factor for *Leishmania* spp. infection (OR = 4.81; 95% CI = 1.23 - 18.75), however, there was no risk factor associated with *T. cruzi* infection. We concluded that dogs attended at veterinary clinics in João Pessoa are exposed to *Leishmania* spp. and *T. cruzi* infections, as well as it is suggested that greater care should be taken for dogs with access to the street.

Keywords: Leishmaniosis, Chagas disease, Urban environment, Domiciled dogs, Northeastern Brazil.

Resumo

O objetivo deste estudo foi determinar a prevalência e os fatores de risco associados à infecção por *Leishmania* spp. e *Trypanosoma cruzi* em cães atendidos em clínicas veterinárias na cidade de João Pessoa, Estado da Paraíba, Nordeste do Brasil. Foram utilizadas 384 amostras sanguíneas de cães procedentes de atendimentos de 34 clínicas veterinárias no período de abril de 2015 a maio 2016. Para diagnóstico sorológico da infecção por *Leishmania* spp. foi empregada a reação de imunofluorescência indireta (RIFI), utilizando promastigotas de *Leishmania major*, adotando ponto de corte 1:40. Os soros reagentes foram submetidos a uma prova confirmatória utilizando o ELISA S7. Os animais foram considerados positivos quando as amostras reagiam para os dois testes. Para o diagnóstico sorológico para doença de Chagas foi realizada a RIFI, com epimastigotas de *T. cruzi* (estirpe Y) adotando ponto de corte 1:40. A soroprevalência para *Leishimania* spp. foi de 2,8% (11/384). Para *T.cruzi* a soroprevalência foi de 1,5% (6/384). Apenas dois animais foram positivos para ambos parasitas. A variável, acesso à rua, foi identificada como fator de risco para infecção por leishmaniose (OR= 4,81; [IC:1,23 -18,75]). Conclui-se que os cães atendidos em clínicas veterinárias de João Pessoa estão expostos às infecções por *Leishmania* spp. e *T. cruzi.* Com base nos fatores de risco, sugere-se maiores cuidados com cães que tem acesso à rua.

Palavras-chave: Leishmaniose, Doença de Chagas, Nordeste

Introduction

Dogs are an important part in the development of society, performing various roles such as work, security and company (CANATTO et al., 2012). Due to the intensification of the relationships between dogs and humans, a great concern arises related to the transmission of diseases; furthermore there are the massive alterations in the environment, such as deforestation and increase of urbanization in previously preserved areas, which causes alteration to the dynamics of these diseases (ZINSSTAG et al., 2011). In this perspective, the canine visceral leishmaniasis (CVL) and the Chagas disease (CD) assume importance because they are endemic zoonosis in Brazil.

The CVL and the CD are anthropozoonosis caused by the protozoa *Leishmania spp*. and *Trypanossoma cruzi*, respectively. In Brazil, the CVL is transmitted by the bite of hematophagous mosquitoes of the genus *Lutzomyia*, and the reservoirs are the wild and domestic canids. The CD, also known American trypanosomiasis, is transmitted mainly by the contact of the susceptible individuals with the feces of hematophagous insects of the *Reduviidae* Family after their blood repast . Other important transmission routes include the oral route by means of the ingestion of contaminated food, and congenitally. The reservoirs of the *T. cruzi* are about 200 domestic and wild mammals (GALVÃO, 2014).

After the 1990's, the presence of these agents was restricted to the rural environment, however, in recent years, due to the increase of the urbanization, mainly the invasion of peri-urban areas, occurred their proliferation in the urban environment, and as a consequence, the number of infected people and domestic animals has been rising (AMORÁ et al., 2006; ARGOLO et al., 2008, WHO, 2010). According to the SINAN, in Brazil, from 2001 to 2015 were diagnosed approximately 50 thousand cases of visceral leishmaniosis in humans, with most cases coming from the Northeast region. It is estimated that in Brazil

about one million people are infected with Chagas disease, with the greatest occurrence in the North region (WHO, 2015).

Recent studies indicated that the seroprevalence of CVL in Brazil varies from 7.8 to 41.7% (BARBOSA et al., 2015; CARVALHO et al., 2015; FERNANDES et al., 2016) having as main risk factors the semi-domiciled and free rearing of dogs, and contact with other animals (CURI et al., 2014; FERNANDES et al., 2016; BRITO et al., 2016). For CD in dogs the prevalence varied from 4.08% to 38% (MENDES et al., 2013; SALDAÑA et al., 2015; BEZERRA et al., 2014FERNANDES t al., 2016), being considered as risk factors the semi-domiciled and free rearing of dogs, contact with bovine, living in mud-houses, poultry houses close to the homes and accumulation of garbage (FERNANDES et al., 2016;).

From the point of view of public health, epidemiological inquiries are tools of upmost importance, specifically for *Leishmania* spp. and *T. cruzi*, regarding the monitoring, distribution and mapping of these diseases, mainly in studies with dogs, besides these animals being reservoirs of these zoonosis they behave as sentinels of the diseases. This way, the objective of this work was to determine the prevalence and risk factors for the infections by *Leishimania* spp. and *Trypanossoma cruzi* in dogs attended at veterinary clinics in João Pessoa, State of Paraíba, Northeast of Brazil.

Material e Methods

Study area

The survey was conducted in the municipality of João Pessoa, capital city of the state of Paraíba, which has about 720 thousand inhabitants (BRASIL, 2010), distributed in 59 boroughs, and with an area of preserved Atlantic Forest in the center of the urban spot and bordered by mangroves (PARAÍBA, 2014). Its climate is humid tropical, with annual average temperatures of 23ºC and annual rainfall rate above 1,906 mm (PEREIRA, 2014).

Sampling

The selection of the veterinary clinics was based on data from the Regional Council of Veterinary Medicine (CRMV-PB), which has informed the existence of 40 registered clinics, however, in the on-the-spot verification four establishments had closed and in two others the owners preferred not to participate. Then, 34 veterinary clinics in the municipality of João Pessoa distributed in the four zones of the city (north, south, east and west) were used.

The minimum number of animals was determined by the formula for simple random sampling (THRUSFIELD, 2004):

$$n = \frac{Z^2 \text{ x } P\ (1 - P)}{d^2}$$

Where:

n = number of sampled animals

Z = value of the normal distribution for the confidence level of 95%

P = expected prevalence of 50% (sampling maximization)

d = error of 5%

In total, 384 apparently healthy dogs, unvaccinated or vaccinated over six months against leptospirosis were selected. Females in the periparturient and lactation periods were excluded from the study. The collections were carried out in the period from April 2015 to May 2016. Samples were collected by venipuncture of the cephalic or jugular vein, using vacuum tubes of 5 mL, and sera were placed into microtubes and frozen until the serologic tests were performed.

Diagnosis for Leishimania ssp and *Trypanossoma cruzi*

For the diagnosis of both agents the indirect immunofluorescence reaction (IFAT) was used, adopting a cut-off point 1:40. The reagent samples were titrated in the dilutions 1:40, 1:80, 1:160, 1:320 and 1:640 according to Camargo's methodology (1966). In the diagnosis of *Leishmania* spp. were used slides impregnated with *Leishmania major* promastigotes and in the diagnosis of *T.cruzi* were used *T. cruzi* epimastigotes (strain Y). Both antigens were maintained in LIT culture medium (Liver Infusion Tryptose) and NNN (Neal, Novy, Nicolle) culture medium. The positive and negative control sera for the parasites were provided by the Diagnostic Laboratory of Zoonoses, of the Veterinary Hygiene and Public Health Department, of the FMVZ/UNESP – Botucatu Campus, SP. The final antibody titer was determined as the one correspondent to the highest dilution of the sera. In these conditions the membranes of at least 50% of the promastigotes (VCL) and epimastigotes (CD) emitted fluorescence at the cut-off point 40 or above (FERNANDES et al., 2016).

The samples which reacted positively both to *Leishmania* spp. and to *T. cruzi* were submitted to a confirmatory test using the ELISA S7 kit (Biogene Indústria & Comercio Ltda ME, Recife-PE, Brazil) for the diagnosis of CVL. The execution of the test was as specified by the manufacturer.

To be considered positive for *Leishmania* spp. the dogs must have been positive in the IFAT with a minimum dilution of 1:40 and simultaneously reactive in the ELISA S7 test. For the determination of animals positive for *T. cruzi* the samples reacted at a minimum dilution of 1:40 in the IFAT. The samples which reacted positively for both agents were identified as co-infections.

Risk factor analysis

Epidemiological questionnaires were applied to dog owners in order to obtain data to be used in the risk factor analysis. The analyzed variables and their respective categories were: educational level of the owner (illiterate, incomplete primary school, complete primary school, incomplete high school, complete high school, incomplete university degree, university degree), gender (female, male), breed (pure-bred, crossbred), age (up to 48 months, 49 to 72 months, above 72 months), access to street (no, yes), feed (commercial food, homemade food, food scraps, raw meat), access to treated water (no, yes), reason for keeping the dog (company, guard, other), contact with dogs (no, yes), contact with bovine (no, yes), contact with equine (no, yes), contact with wild animals (no, yes), contact with cats (no, yes), contact with goats (no, yes), contact with sheep (no, yes), contact with swine (no, yes), type of housing (masonry, shacks, stilts, mud huts), where the animal is kept (soil, cement, soil/cement), environment where the animal lives (rural or

urban), environment near woodlands or agricultural areas (no, yes), regular garbage collection (no, yes), cleaning and disinfection of the environment (daily, weekly/fortnightly, monthly), vaccination (no, yes), contact with flooded areas (no, yes), presence of rodents (no, yes), occurrence of abortions (no, yes), occurrence of stillbirths (no, yes), presence of ticks (no, yes).

An univariable exploratory analysis of the data was carried out for the selection of variables with $P \leq 0.2$ using the chi-square or Fischer exact tests. Subsequently the significant variables passed to a multivariable analysis using the multiple logistic regression with significant level of 5% (HOSMER; LEMESHOW, 2000). The adjustment of the final model was verified with the Hosmer and Lemeshow test, by which a value of $P \geq 0.05$ indicates a good fit. The collinearity between independent variables was verified by correlation analysis; for the variables with strong collinearity (correlation coefficient > 0.9), one of the two variables was excluded from the multiple analysis according to the biological plausibility (DOHOO et al., 1997). Confounding was evaluated by the monitoring the alterations in the model's parameters (> 20%) when adding new variables. Data analyses were carried out using the SPSS 23.0 *for* Windows software.

Results

Of the 384 evaluated samples 21(5.4%) were positive only for IFAT, however only eleven were confirmed for *Leishmania* spp. in the Elisa S7 which generated a prevalence of 2.8%. The antibody titer for *Leishmania* spp. of the positive samples in both tests and their respective frequencies in the IFAT were 40 (22.2%), 80 (33.3%), 160 (33.3%) and 640 (10.2%). Six of the tested samples (1.5%) were positive for *T. cruzi*. The antibody titers and their frequencies were 40 (25%), 80 (50%) and 320 (25%). Two samples were positive for both agents with titers of 40 for *Leishmania* spp. and 80 for *T. cruzi*. In Figure 1 are presented maps of the municipality of João Pessoa and the spatial distribution per residence of the dogs positive and negative for *Leishmania* spp. (A) and *T. cruzi* (B). In the univariate analysis of the risk factors for *Leishmania* spp., the following variables were selected: age of the animals , type of rearing, access to the street, contact with flooded areas, occurrence of stillbirths (Table 1) and in the multivariate analysis the variable access to the street was identified as a risk factor (OR= 4.81; CI 95%=1.23-18.75; *P*= 0.024). Risk factors for CD were not identified.

Discussion

Domestic dogs are reservoirs of the CVL and play an important role in the transmission of the agent in endemic areas such as the Brazilian Northeast. Recent studies about the prevalence of CVL in this region have presented variations between 7.8% to 41.7% (SANTOS et al., 2010; BARBOSA et al., 2015; CARVALHO et al., 2015; FERNANDES et al., 2016).

The prevalence value of the present study was lower than the aforementioned, and this possibly occurred due to the living conditions of the animals, seen that all the participants were from veterinary clinics and domiciled . Thus, it is assumed that the owners of these animals have greater care with their health and hygiene. A concerning issue is that all the animals seropositive for CVL in this study did not

present clinical signs compatible to the disease, therefore it is important to highlight that the presence of asymptomatic seropositive individuals is a strong indicator of the maintenance of the parasite in the municipality.

According to the Figure 1 it is observed that the canine visceral leishmaniosis is geographically distributed in all the regions of the municipality of João Pessoa. Notwithstanding, many of these areas of the city suffered a rapid urbanization characterized by the deforestation of preserved areas and its transformation into residential sectors. Santos et al. (2016) using information from patient's records from the University Hospital of the Federal University of Paraiba, in the years 2007 to 2012, stated that in this period 50 people contracted Visceral leishmaniosis in the municipality of João Pessoa and that the majority of the cases concentrated in these two regions mainly in the boroughs of Mangabeira, Oitizeiro and Alto do Mateus.

Chagas disease is also endemic in dogs in the Brazilian northeast presenting prevalence of 4.08% to 38% (MENDES et al., 2013; BEZERRA et al., 2014; FERNANDES et al., 2016; PEREZ et al., 2016). It is important to highlight that CD is an anthropozoonosis which presents a predominant rural occurrence and that the majority of the studies mentioned above were carried out with animals originated from these areas. This fact may explain the low prevalence of CD in the present study, as all the dogs evaluated were originally from an urban environment. According to data from the SINAN in Paraiba in the period from 2001 to 2006 were notified 110 cases of acute CD in humans. Evaluating this same database between the years of 2007 to 2014 only one case was diagnosed, which leads to believe that the disease is underdiagnosed in the state, what may be confirmed by the data of Costa (2014) who evaluated human beings in the municipality of Santo André, Paraiba, whose prevalence of antibodies anti-*T. cruzi* was of 3.2%.

In the present work were observed two animals positive for both agents. It is known that the state of Paraiba is endemic for CVL and presents confirmed cases of Chagas disease in dogs (BRITO-FILHO, 2013; SANTANA et al., 2014). Therefore the is a possibility of simultaneous occurrence of both infections as observed by Fernandes et al. (2016) in Natal, Rio Grande do Norte, and Morais et al. (2013) in Araguaína, Tocantins. However, Luciano et al. (2009), who evaluated the occurrence of cross reactions between *Leishmania* spp. and *T. cruzi* in the IFAT in dogs, stated that the occurrence of cross reactions for these agents are common , and it is possible to identify cross reactions by the analysis of the titres . The levels of antibodies which the animals presented, in the present study, for *Leishmania* spp. and *T. cruzi* were 40 and 80 respectively. Thus as there are no great discrepancies between the titres it is believed that the animals evaluated had the two coexisting infections (LUCIANO et al., 2009).

Access to the street was a risk factor for the infection by *Leishmania* spp. In dogs in João Pessoa. Its occurrence may be justified by the greater possibility of exposure to the vectors. The presence of humid environments rich in organic matter, in the municipality, favors the reproduction and dissemination of the insects which transmit leishmaniosis. Furthermore, the rapid urbanization contributes for the occurrence of the disease in animals and humans. The cases of seropositive dogs contribute to the occurrence of the disease in animals and humans. The cases of dogs found positive for CVL and CD (Figure 1) were concentrated in areas close to woods or areas which recently were deforested and were transformed into housing sectors. It

deserves to highlighting, as it is known that the infections in animals precede their emergence in humans (QUINNELL; COURTENAY, 2009).

Visceral leishmaniosis and CD in dogs and humans are becoming more and more frequent in the urban centers, mainly due to environmental and socioeconomic imbalances. The results of this research are very important for the city of João Pessoa, as over the last decades the municipality has been undergoing rapid urbanization, which alerts for the need for more complex, multidisciplinary and multisystemic approaches as the "One health", particularly when dealing with zoonotic diseases which have a strong environmental component involved (CUNNINGHAM et al., 2017; CLEVELAND et al., 2017).

Conclusion

It is concluded that the dogs attended at veterinary clinics of João Pessoa are exposed to infections by *Leishmania* spp. and *T. cruzi,* which raises concern from the public health point of view, as they are important zoonosis with the involvement of environmental components. Based on the risk factors, it is suggested that greater care should be taken with dogs which have access to the streets.

References

AMORA, S.S.A.; SANTOS, M.J.P.; ALVES, N.D.; COSTA, S.C.G.; CALABRESE, K.S.; MONTEIRO, A.J.; ROCHA, M.F.G. Fatores relacionados com a positividade de cães para leishmaniose visceral em áreas endêmicas do Estado do Rio Grande do Norte, Brasil. *Ciências Rural*, Santa Maria, v. 36, n. 6, p. 1854-1859, 2006.

ARGOLO, A.M.; FELIX, M.; PACHECO, R.; COSTA J. *Doença de Chagas e seus Principais Vetores no Brasil*. Rio de Janeiro: Imperial Novo Milênio/Fundação Oswaldo Cruz, 2008. 63p.

BARBOSA, I.R.; CARLOTA, F.C.; DE ANDRADE-NETO, V.F. Seroepidemiological survey of canine leishmania infections from peripheral areas in Natal, Northeast Brazil. *The Open Microbiology Journal*, Hilversum, v. 9, n.1, p. 43–47, 2015.

BEZERRA, C.M.; CAVALCANTI, L.P.G.; SOUZA, R.C.M; BARBOSA, S.E; XAVIER, S.C.C; JANSEN, A.M; RAMALHO, R.D.; DIOTAIUT, L. Domestic, peridomestic and wild hosts in the transmission of *Trypanosoma cruzi* in the Caatinga area colonised by *Triatoma brasiliensis*. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, v. 109, n.7, p. 887-898, 2014.

BRITO, F.G.; LANGONI, H.; SILVA, R.C.; ROTONDANO, T.E.F.; MELO, M.A.; PAZ, G. S. Canine visceral leishmaniasis in the Northeast Region of Brazil. *The journal of venomous animals and toxins*

including tropical diseases, Botucatu, v.*22*, n.15, 2016. <http://doi-org.ez42.periodicos.capes.gov.br/10.1186/s40409-016-0069-4> acesso em: 07 jul. 2017.

CAMARGO ME. Fluorescent antibody test for the sorodiagnosis of American trypanosomiasis: technical modification employing preserved culture forms of in a slide test. *Trypanosome cruzi*. *Revista Instituto de Medicina Tropical*, São Paulo v.8, n.5, p.227-235, 1966.

CANATTO, B.D.; SILVA, E.A.; BERNARDI, F.; MENDES, M.C.N.C.; PARANHOS, N.T.; DIAS, R.A. Caracterização demográfica das populações de cães e gatos supervisionados do município de São Paulo. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v.64, n.6, p.1515-1523, 2012.

CARVALHO, F.S.; WENCESLAU, A.A.; ALBUQUERQUE, G.R.; MUNHOZ, A.D.; GROSS, E.;. CARNEIRO, P.L.S.; OLIVEIRA, H.C.; ROCHA, J.M.; SANTOS, I.A.; REZENDE, R.P. *Leishmania* (*Viannia*) *braziliensis* in dogs in Brazil: epidemiology, co-infection, and clinical aspects. *Genetics and Molecular Research*, Ribeirão Preto, v.14 n.4, p. 12062-12073, 2015.

COSTA, K.Q. *Avaliação soroepidemiológica da Doença de Chagas no município de Santo André-PB*. 2014. Monografia (Trabalho de conclusão de curso em Ciências Farmacêuticas) - Universidade Federal da Paraíba, João Pessoa.

CURI, N.H.A.; PASCHOAL, A.M.O.; MASSARA, R.L.; MARCELINO, A.P.; RIBEIRO, A.A.; PASSAMANI, M.; CHIARELLO, A.G. Factors Associated with the Seroprevalence of Leishmaniasis in Dogs Living around Atlantic Forest Fragments. *Plos one*, São Francisco, v.9, n.8, 2014. Disponível em: <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0104003> Acesso em: 07 jul. 2017

DOHOO, I.R.; DUCROT, C.; FOURICHON, C.; DONALD, A.; HURNIK, D. An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. *Preventive Veterinary Medicine*, Amsterdam, v.29, n.3, p.221-239, 1997.

FERNANDES, A.R.F.; PIMENTA, C.L.R.M.; VIDAL, I.F.; OLIVEIRA, G.C.; SARTORI, R.S; ARAÚJO, R.B.; MELO, M.A.; LANGONI, H.; Azevedo, S.S.. Risk factors associated with seropositivity for *Leishmania* spp. and *Trypanosoma cruzi* in dogs in the state of Paraiba, Brazil. *Revista Brasileira de Parasitologia Veterinária*, Jaboticabal, v.*5*, n.1, p.90-98, 2016.

GALVÃO, C. *Vetores da doença de Chagas no Brasil*. Curitiba: Sociedade Brasileira de Zoologia, 2014. 291p.

GOVERNO DO ESTADO DA PARAÍBA (PARAÍBA, PB). *Estudo para subsidiar a criação de unidade de conservação de proteção integral da mata do buraquinho – Paraíba*. João Pessoa, 2014.

HOSMER, D.W.; LEMESHOW S. *Applied Logistic Regression.* New York: John Wiley & Sons, 2000. 397p.

MENDES, R.S.; SANTANA, V.L.; JANSEN, A.M.; XAVIER, S.C.C.; VIDAL, I.F.; ROTONDANO, T.E.F.; SOUZA, A.P. Aspectos epidemiológicos da Doença de Chagas canina no semiárido paraibano. *Pesquisa Veterinária Brasileira,* Rio de Janeiro, v. 33, n.12, p. 1459-1465, 2013.

PEREIRA, M. D. B. *As chuvas na cidade de João Pessoa: uma abordagem genética*. 2014. Monografia (Trabalho de conclusão de curso em Curso de Geografia) - Universidade Federal da Paraíba, João Pessoa.

PEREZ, T.D.; FIGUEIREDO, F.B.; JUNIOR, A.A.M; SILVA, V.L.;, MADEIRA, M.F; BRAZIL, R.P; COURA, J.R.. Prevalence of american trypanosomiasis and leishmaniases in domestic dogs in a rural area of the municipality of São João do Piauí, Piauí state, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo,* São Paulo, v. 58, 79, 2016. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S003646652016005000261&lng=en&nrm=iso&tlng=en> acesso em: 07 jul. 2017.

QUINNELL, R.J.; COURTENAY ,O. Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology*. London, v.136, n. 14, p.1915–1934, 2009.

SALDAÑA, A.; CALZADA, J. E.; PINEDA, V.; PEREA, M.; RIGG, C.; GONZÁLEZ, K.; MARIA SANTAMARIA,M.; GOTTDENKER,N.L.; CHAVES, L. F. Risk factors associated with *Trypanosoma cruzi* exposure in domestic dogs from a rural community in Panama. *Memórias Do Instituto Oswaldo Cruz*, Rio de janeiro, v.*110, n.* 7, p.936–944, 2015.

SANTOS, J.M.L.; DANTAS-TORRES, F.; MATTOS, M.R.F.; LINO, F.R.L.; ANDRADE, L.S.S.; SOUZA, R.C.A.; BRITO, F.L.C.; BRITO, M.E.F.; BRANDÃO-FILHO, S.P.; SIMÕES-MATTOS, L. Prevalência de anticorpos anti-*Leishmania* spp em cães de Garanhuns, Agreste de Pernambuco. *Revista da Sociedade Brasileira de Medicina Tropical*, Rio de Janeiro, v.43, n. 1, p. 41-45, 2010.

THRUSFIELD, M. *Epidemiologia Veterinária*. 2.ed. São Paulo: Roca, 2004. 556p.

WORLD HEALTH ORGANIZATION – WHO. Chagas disease (American trypanosomiasis) [online] <disponível em: http://www.who.int/mediacentre/factsheets/fs340/en/. Acesso em: 18 set. 2017.

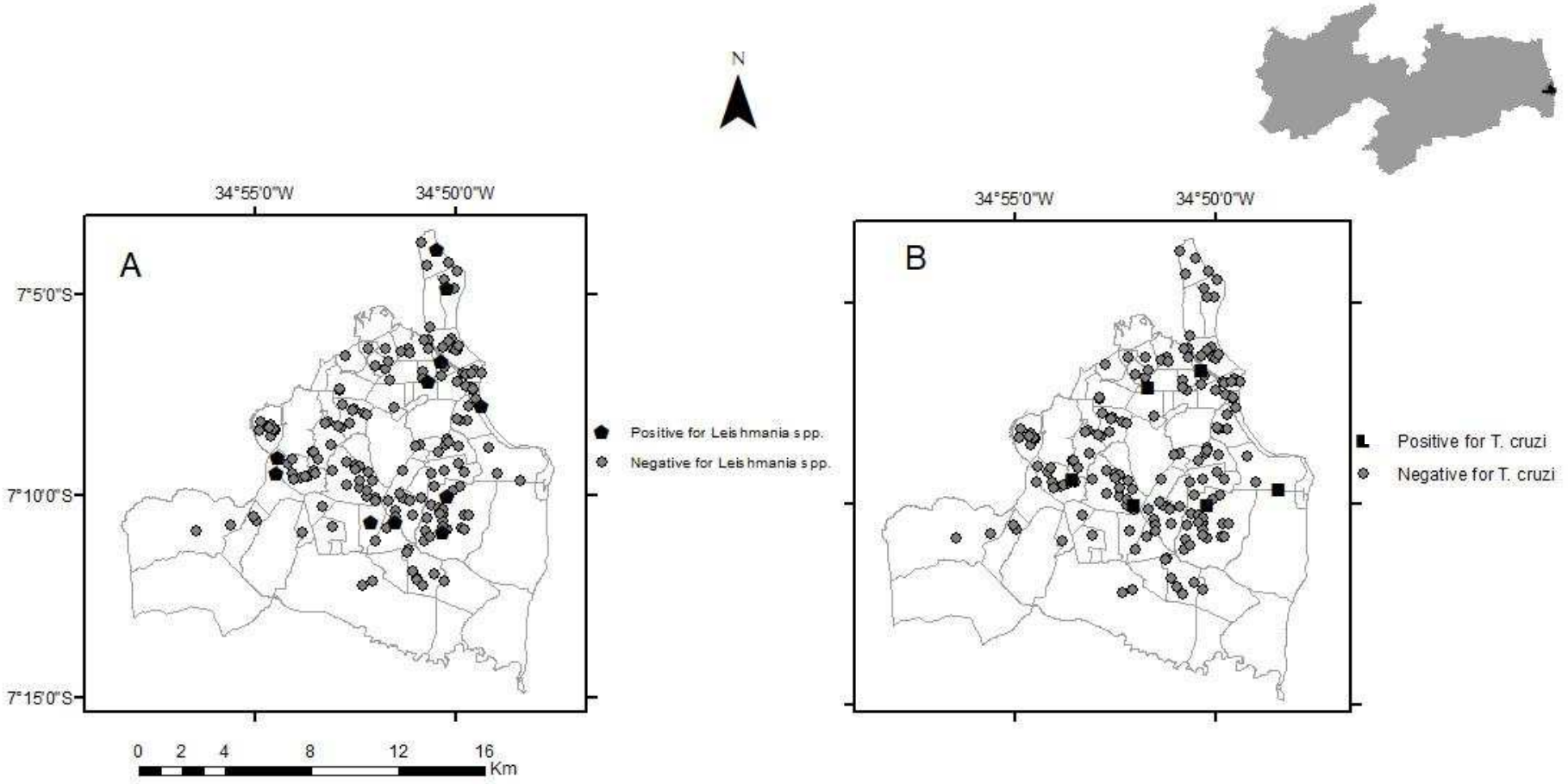
WORLD HEALTH ORGANIZATION – WHO Chagas disease: control and elimination, 2010. [online]. <Disponível em**:** http://apps.who.int/gb/ebwha/pdf_files/WHA63/A63_17-en.pdf**.>** Acesso em: 18 set. 2017

ZINSSTAG, J.; SCHELLING, E.; WALTNER-TOEWS, D.; TANNER, M. From “one medicine” to “one health” and systemic approaches to health and well-being. *Preventive Veterinary Medicine*, Amsterdam, v.101, n. 3-4, p.148–156, 2011.

Table 1. Univariate analysis of risk factors associated with canine leishmaniasis in the municipality of João Pessoa (*P*≤0,2).

Variable	Categories	Total nº of animals	Nº seropositive animals (%)	*P*
Age	Up to 48 months	219	5 (2,3)	
	49 to 72 months	63	4 (6,3)	
	Above 72 meses	102	2 (2,0)	0,191
Access to street	No	238	3(1,3)	
	Yes	146	8(5,5)	0,024
Contact with flooded areas	No	356	9(2,5)	
	Yes	28	2(7,1)	0,188
Occurrence of stillbirths	No	377	10(2,7)	
	Yes	7	1(14,3)	0,185

Figure 1. Distribution of dogs positive for Leishmania spp. (A) and Trypanosoma cruzi (B) attended at veterinary clinics in the city of João Pessoa, according to their housing, from April 2015 to May 2016.



6. CONCLUSÕES

Ao final desta tese pode-se concluir que:

- ✓ Foram detectados cães sorologicamente reagentes para os agentes patogênicos *Leptospira* sp, *Toxoplasma gondii*, *Neospora caninum*, *Leishmania* spp. e *Trypanossoma cruzi* atendidos em clínicas veterinárias na cidade de João Pessoa, Paraíba.
- ✓ A detecção desses agentes nos cães domiciliados de João Pessoa é preocupante uma vez que esses animais podem estar sendo reservatórios de graves zoonoses, o que aumenta o risco de transmissão dessas enfermidades, sobretudo nas comunidades em vulnerabilidade social.
- ✓ Baseado nas análises de fatores de risco observou-se que são necessários maiores cuidados com a higiene do local onde os cães vivem, deve se ter mais atenção com os animais que habitam áreas próximas a matas, além disso, deve-se controlar o acesso dos animais a rua.
- ✓ Sugere-se que para as reduções das prevalências dos agentes infecciosos encontrados sejam adotadas politicas públicas baseadas na correção dos fatores de risco indentificados nas visões multidisciplinares dos conceitos de saúde única.