NARIANNE FERREIRA DE ALBUQUERQUE

O PAPEL DAS CAPIVARAS (*Hydrochoerus hydrochaeris* LINNAEUS, 1766) COMO PORTADORAS DE LEPTOSPIRAS EM ÁREA URBANA E RURAL NA AMAZÔNIA OCIDENTAL

Dissertação apresentada à Universidade Federal do Acre, como parte das exigências do Programa de Pós-Graduação em Sanidade e Produção Animal Sustentável na Amazônia Ocidental, para a obtenção do título de Mestre em Ciência Animal.

RIO BRANCO
ACRE – BRASIL
SETEMBRO – 2016

Ficha catalográfica elaborada pela Biblioteca Central da UFAC

A345p Albuquerque, Narianne Ferreira de, 1991-

O papel das capivaras (*Hydrochoerus hydrochaeris* Linnaeus, 1766) como portadoras de Leptospiras em área urbana e rural na Amazônia Ocidental / Narianne Ferreira de Albuquerque. – 2016.

32 f.: il.; 30 cm.

Dissertação (Mestrado) – Universidade Federal do Acre, Programa de Pós-Graduação em Sanidade e Produção Animal Sustentável na Amazônia Ocidental, 2016. Incluem referências bibliográficas e apêndices. Orientador: Prof.ª Dra. Luciana dos Santos Medeiros.

1. Zoonoses. 2. Enterobactérias. 3. Leptospiras. I. Título.

CDD: 616.993

Bibliotecária: Maria do Socorro de Oliveira Cordeiro CRB-11/667

NARIANNE FERREIRA DE ALBUQUERQUE

O PAPEL DAS CAPIVARAS (*Hydrochoerus hydrochaeris* LINNAEUS, 1766) COMO PORTADORAS DE LEPTOSPIRAS EM ÁREA URBANA E RURAL NA AMAZÔNIA OCIDENTAL

Dissertação apresentada à Universidade Federal do Acre, como parte das exigências do Programa de Pós-Graduação em Sanidade e Produção Animal Sustentável na Amazônia Ocidental, para obtenção do título de Mestre em Ciência Animal.

APROVADA: 02 de setembro de 2016.

Dra. Clarice Maia Carvalho
Universidade Federal do Acre

Dra. Vânia Maria França Ribeiro
Universidade Federal do Acre

Dra. Luciana dos Santos Medeiros
UFAC
(Orientadora)

Aos meus familiares e amigos.

Dedico.

AGRADECIMENTOS

A Deus Pai, Criador e Autor da vida. Seu amor e cuidado sempre me acompanham.

À minha mãe, Maria Santíssima, que passou a frente em todas as situações e por sua intercessão.

Aos meus pais Marcos Aurélio Apolinário de Albuquerque e Maria Barbosa Ferreira que sempre apoiaram as minhas decisões, pelo suporte, conselhos e consolos nas mais diversas situações da minha vida.

À minha orientadora, Dra. Luciana dos Santos Medeiros, por sua disponibilidade, paciência, confiança e amizade. Seus ensinamentos foram além do científico.

À Dra. Maria Vânia França Ribeiro, que idealizou o projeto de estudo de enfermidades em capivaras, agradeço a oportunidade de fazer parte da realização desse projeto.

Aos meus colegas do Mestrado Itacir Farikoski, Karla Gruhn e Paula Joseanny, pela ajuda e união em todos os momentos, principalmente durante as coletas, não foi fácil mas conseguimos!

Aos graduandos em Medicina Veterinária, os quais foram fundamentais, sempre dispostos a ajudar.

À UFAC (Universidade Federal do Acre) e ao PPGESPA (Programa de Pós-graduação em Sanidade e Produção Animal Sustentável na Amazônia Ocidental) pela oportunidade.

À UFF (Universidade Federal Fluminense) na pessoa do Dr. Walter Lilenbaum e toda a equipe do Laboratório de Bacteriologia Veterinária da UFF (Gabriel Martins, Ana Paula Loureiro, Anahi Souto e Priscila da Silva) pela parceria firmada, apoio teórico e prático nas análises das amostras.

A todos que de alguma forma fizeram parte desse projeto.

"*A fé e a razão caminham juntas,*
mas a fé vai mais longe."

Santo Agostinho (354-430)

CERTIFICADO DO COMITÊ DE ÉTICA NO USO DE ANIMAIS – UFAC

Título do projeto: A capivara (*Hydrochoerus hydrochaeris*) da Amazônia Ocidental como possível reservatório de agentes etiológicos causados por zoonoses.

Processo número: 23107.016723/2014-41.

Protocolo número: 34/2014.

Responsável: Prof. Dra. Vânia Maria França Ribeiro.

Data de aprovação: 01/01/2014

RESUMO

ALBUQUERQUE. Narianne Ferreira de. Universidade Federal do Acre, setembro de 2016. **O Papel das capivaras** (*Hydrochoerus hydrochaeris)* **como portadoras de leptospiras em área urbana e rural na Amazônia Ocidental.** Orientadora: Luciana dos Santos Medeiros. A leptospirose em capivaras tem sido descrita geralmente baseada em evidências sorológicas, enquanto que a cultura bacteriana é pouco relatada. Na Amazônia Ocidental, ela se apresenta como endêmica e de alta soroprevalência em diferentes espécies de animais silvestres, domésticos e seres humanos. O presente estudo teve como objetivo investigar o papel das capivaras como portadoras de leptospiras em áreas urbanas e rurais na Amazônia Ocidental. Um total de 44 animais foram capturados e 41 amostras de sangue para sorologia e 41 amostras de urina para PCR e cultura bacteriana foram obtidas. Um total de 18/41 (43,9%) de soros foram reativos. A maioria dos títulos foram baixos, o que indica uma infecção crônica. A PCR foi positiva em 13/41 (31,7%) amostras. Foram recuperados oito isolados a partir de amostras de urina, seis deles pertenceram ao sorogrupo Icterohaemorrhagiae, um para Grippotyphosa e um para Shermani. Pode ser notado um elevado número de portadoras (pela PCR) e uma tendência para abrigar sorovares do sorogrupo Icterohaemorrhagiae. Estes resultados sugerem que as capivaras são infectadas por leptospiras. Em comparação com ratos, capivaras apresentam infecção, com títulos baixos e eliminação bacteriana a longo prazo, sendo assim ela pode estar agindo como reservatório dessa bactéria.

Palavras-chaves: Isolamento, Roedores, Animais Selvagens.

ABSTRACT

ALBUQUERQUE. Narianne Ferreira de. Universidade Federal do Acre, September 2016. **The role of capybaras (*Hydrochoerus hydrochaeris*) as carriers of leptospires on periurban and rural areas on Western Amazon**. Advisor: Luciana dos Santos Medeiros. Leptospirosis on capybaras has been described, usually based on serological evidences, while bacterial culture has been scarcely reported. It was reported to be endemic in Western Amazon, and high seroprevalence have been reported in different species, such as wildlife, domestic animals and human beings. The present study aimed to investigate the role of capybaras as carriers of leptospires on periurban and rural areas on Western Amazon. A total of 44 animals were trapped and 41 blood samples for serology and 41 urine samples for PCR and bacterial culture were obtained. A total of 18/41 (43.9%) of sera were reactive, and titres were generally low, indicating a chronic infection. PCR was positive on 13/41 (31.7%) samples, while a total of eight isolates could be recovered from urine samples, six of them belonging to serogroup Icterohaemorrhagiae, one to Grippotyphosa and one to Shermani. A high number of carriers (by PCR) and a tendency for harboring strains of serogroup Icterohaemorrhagiae could be noticed. Our results suggest that capybaras are infected by leptospires. In analogy to Norway rats, capybaras present chronic infection, with low titres and long-term bacterial shedding, and may be acting as reservoirs of that bacterium.

Keywords: Isolation, Rodents, Wildlife.

SUMÁRIO

págs.

1 ARTIGO

1.1 Artigo 1

The role of capybaras (*Hydrochoerus hydrochaeris*) as carriers of leptospires on periurban and rural areas on Western Amazon.

Narianne Ferreira de Albuquerque, Gabriel Martins, Luciana Medeiros, Walter Lilenbaum e Vânia Maria França Ribeiro.

Submetido à Acta Tropica em Abril de 2016.

1. Introduction

Leptospirosis is an infectious zoonotic disease determined by bacteria of *Leptospira* genus. It happens on well-defined rural and urban cycles (Haake and Levett, 2015). On the urban cycle of the disease, rodents, mainly but not exclusively *Rattus norvegicus*, are known as reservoirs of the bacterium. Other rodents have also been recognized as reservoirs, as *Rattus rattus*, *Mus musculus* (Fortes-Gabriel et al., 2016), and the wild species *Cavia aperea* (Monte et al., 2013), *Arvicola* sp., *Crocidura* sp., *Talpa* sp., *Sorex* sp., and *Microtus* sp. (Obiegala et al., 2016).

The capybara (*Hydrochoerus hydrochaeris*) is the largest living rodent in the world. It occurs on Latin America from Panama to Uruguay (García-Esponda and Candela, 2016). That species requires abundant and permanent water supply for its living (Alho and Rondon, 1987). Its role as reservoirs of other pathogens has been reported, as *Toxoplasma* sp. (Abreu et al., 2016), *Trypanosoma* sp. (Da Silva et al., 2016), and *Rickettsia* sp. (Monje et al., 2015).

Leptospirosis on capybaras has been described on various regions of Brazil, usually based on serological evidences (Silva, et al., 2009; Chiacchio et al., 2014; Langoni, et al., 2016). In these studies, capybaras presented 26-41.2% of seropositivity, and serogroups Australis, Canicola, Tarassovi, Icterohaemorrhagiae and Pomona have been reported. Nevertheless, serology cannot be considered as a reliable tool for diagnosing the infection, since it may indicate simple exposure to the agent. The gold-standard diagnostic method, bacterial culture, has been scarcely reported on capybaras, and points out that species as a potential source of infection. The National Collection of Leptospires of Animal Origin (www.labv.uff.br) refers to only ten strains ever recovered from

that rodent, being seven from serogroup Grippotyphosa (Marvulo et al., 2002), two from serogroup Shermani (S.A.Vasconcellos, personal communication) and a single one from serogroup Icterohaemorrhagiae (Jorge et al., 2012).

Amazon is the largest rain forest in the world and in the past decades human population has enormously increased in that region. It presents equatorial climate (Af and Am by Koeppen classification) and a close proximity between cities and forest (Saleska et al., 2016). Rio Branco is the biggest city on Western Amazon (population around 400,000) and is surrounded, by one side, by extent areas of Amazonian forest, and by the other side, by rural areas of cattle breeding. Leptospirosis was reported to be endemic in Western Amazon (Chiebao et al., 2015), and high seroprevalence have been reported in different species, such as wildlife and domestic animals (Jori et al., 2009; Furtado et al., 2015); and human beings (Donaires et al., 2012).

Considering this, the present study aimed to investigate the role of capybaras as carriers of leptospires on periurban and rural areas on Western Amazon.

2. Material and Methods

Handling procedures agreed with Ethical Principles in Animal Research adopted by the Animal Ethic Committee of the Federal University of Acre (process number 23107.016723/2014-41) and were in full compliance with federal permits issued by the Brazilian Ministry of the Environment (License SISBIO number 44791-1).

2.1. Study Area

All the studied regions were located around Rio Branco, capital of the state of Acre, on Western Amazon. Periurban regions were represented by a borderline recently occupied area of the city, contiguous to Amazon forest. Rural regions comprised two different farms located 15 km from the city. In both areas, animals could circulate from the forest to the studied area.

2.2. Animals

The capture of the animals was performed by corral-traps, with daily food. Upon entry into the trap, it was closed and the animals were mechanically contained in dip nets. After that, those animals were identified by microchip and anesthetized with azaperone (1.0 mg/kg), ketamine (12 mg/kg) and diazepam (0.1 mg/kg) intramuscularly (King et al., 2010). A total of 44 capybaras were captured, 21 from rural and 23 from periurban areas. All the captured animals were rigorously examined by veterinarians and no symptoms of clinical leptospirosis (acute disease) were observed.

2.3. Sampling

From the 44 animals, three blood samples presented hemolysis and were not included in this study, one from rural and two from periurban areas. Thus, a total of 41 blood samples were studied. Sampling occurred by puncture of the femoral vein (Vacutainer®, BD, Franklin Lakes, NJ, USA), transported to the laboratory and centrifuged. Serum samples were labelled and stored in 1.5 mL microtubes (Eppendorf®, São Paulo, SP, Brazil) at -20º C to be tested as a batch.

Three animals presented empty bladder and sampling of urine was not possible, one from rural and two from periurban areas. Thus, it was possible to obtain 41 urine samples, collected by cystocentesis that were chilled and transported to the laboratory in syringes. Aliquot of urine samples were used for bacteriological culturing and PCR.

2.4. Serology (MAT)

For detection of anti-*Leptospira* antibodies, Microscopic Agglutination Test (MAT) was performed with a complete panel including 28 serovars representing 24 serogroups (from Institut Pasteur, Paris, France), according to international standards (OIE, 2014). Infective serogroup was considered to be that that presented the highest titre, and animals were considered as seroreactive when presented titres ≥100.

2.5. PCR

DNA was extracted from the urine using the Promega Wizard SV Genomic DNA Purification System® (Promega, Madison, USA). PCR methodology was performed as Hamond et al. (2014) and targets the *lipL32* gene, which is referred to be present only in pathogenic leptospiras (lipL32_45F - 5'AAG CAT TAC TTG CGC TGG TG 3' and lipL32_286R - 5'TTT CAG CCA GAA CTC CGA TT 3').

2.6. Bacterial culturing and serological characterization of the isolates

Few drops of urine were seeded into two tubes containing 5 mL of EMJH (BD Difco, Franklin Lakes, NJ, USA), and two tubes with 5 mL of EMJH supplemented with antimicrobial cocktail STAFF (EMJH-STAFF ; Loureiro et al., 2015) and two tubes containing 5 mL of Fletcher (BD Difco, Franklin Lakes, NJ, USA). Cultures were incubated at 28 ºC and evaluated by dark field microscopy weekly for 30 weeks.

Obtained isolates were tested by Microscopic Agglutination Test (MAT), against a panel of rabbit antisera of 32 reference serovars representing 24 serogroups (provided by Royal Tropical Institute - KIT, Amsterdam), as recommended (Haake and Levett, 2015).

2.7. Statistics

The statistical analysis was performed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Serological and molecular data were treated by Chi-square test and Fisher's exact test. A value of $P<0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Serology (MAT)

A total of 18/41 (43.9%) of sera were reactive. Reactivity was 10/20 (50%) on rural areas and 8/21 on periurban areas (38.1%), a non-significant difference. Seroreactivity was very similar than that described in a recent study

(Langoni et al., 2016) conducted in capybaras from commercial and experimental breeding facilities in Southeast region, Brazil (41.3%). In contrast, a study conducted in free-ranging capybaras from a park in São Paulo, Southeast of Brazil reported a seroreactivity level of 26% (Chiacchioe et al., 2014), while another one reported seroprevalence of 27.3% in capybaras from a slaughterhouse in South region, Brazil (Silva et al., 2009). Noteworthy that, due to the paucity of studies regarding leptospirosis in capybaras, a wide comparison with the literature is difficult to perform.

Amazonian environmental conditions are highly favorable for maintenance of leptospires (Jori et al., 2009). Thus, it was not surprising the high seroreactivity observed in animals of the present study, as well as in other studies conducted in wildlife and domestic species from Amazon biome, such as collared peccary (*Tayassu tajacu* - 86.4%), maned wolves (*Chrysocyon brachyurus* - 75.0%), manatees (*Trichechus inunguis* - 31.1%), cattle (73.6%) and dogs (37.5%) (Deem and Emmons, 2005; Jori et al., 2009; Mathews et al., 2012; Furtado et al., 2015).

Except for two rural animals, that presented titres of 800 and 400, what probably indicates recent infection, titres were generally low, indicating a chronic infection. Half of the reactive sera presented titres of 100, while 38.9% presented titres of 200. In this context, a massive presence of asymptomatic capybara with low titres in the present study was not an unexpected result.

In relation to the serogroup distribution at MAT, Shermani, Pomona, and Icterohaemorrhagiae were predominant, although reactions against Bataviae and Australis were also detected. On rural areas, Icterohaemorrhagiae was predominant, while on periurban reactions against Pomona and Shermani were

the most common. Despite the slight predominance of some serogroups, those differences were also non-significant. It is important do highlight that there is no consensus of predominant serogroups reported by serology in capybaras worldwide. In general, independently of studied scenario (urban, rural, free-ranging animals), a wide gamma of serogroups is reported, impairing comparisons. Additionally, the majority of those studies occurred by convenience sampling, using a non-representative population of animals. Thus, inferences regarding predominant serogroups in studied regions are very limited. Despite that, in Peru the most frequent serogroups reported in capybaras were Sejroe, Grippothyphosa, Mini, Canicola, Ballum and Pyrogenes (Cueva et al., 2010; Muñoz et al., 2014); while in Brazil were Icterohaemorrhagiae, Australis, Pomona, Djasiman and Castellonis (Silva et al., 2009; Langoni et al., 2016).

3.2. Urinary PCR

Considering the 41 urine samples, 13 were positive (31.7%), being seven from periurban (53.8%) and six from rural animals (46.2%), once more a non-significant result. Those results indicate that those animals may be acting as important carriers of that bacterium in both areas, being source of infection to environment and other species. In the present study, we observed that seroreactivity presented low correlation between positivity in urinary PCR. That phenomenon also occurs in domestic animals, and seems to be common in leptospirosis (Hamond et al., 2014).

3.3. Bacterial culturing and serological characterization of the isolates

A total of eight isolates could be recovered from urine samples (19.5%), five (62.5%) from periurban and three (37.5%) from rural areas, a non-significant difference. Serogrouping of the isolates identified six of them as belonging to serogroup Icterohaemorrhagiae (75%), one to serogroup Grippotyphosa (12.5%) and one to serogroup Shermani. Even using selective media, recovery of *Leptospira* is a laborious and low sensitive technique (Haake and Levett, 2015). Additionally, under field conditions that sensitivity seems to be severally impaired (Loureiro et al., 2015). Thus, the high rate of leptospires recovered from urine samples in the present study is a remarkable outcome, since it represents an advance for epidemiological interpretations.

It is also remarkable the massive recovery of isolates belonging to serogroup Icterohaemorrhagiae in both areas. It is well-known that Norway rats play an important role as reservoir of strains from that serogroup in urban scenarios (Panti-May et al., 2016). Nevertheless, the real role of other rodents, including capybaras, as carriers of leptospires in rural or free-ranging scenarios remains to be elucidated. Recently, another study conducted in South of Brazil reported the isolation of a strain of that serogroup in a capybara admitted to the Wildlife Rehabilitation Nucleus (Jorge et al., 2012), what may reinforce the concept of capybaras as reservoirs of that leptospiral strain.

3.4. Final considerations

It is interesting to highlight that, considering serology, bacterial culture and PCR results there was no significant difference between periurban or rural animals. It suggests that, rather than an environmental occasional contamination, capybaras are probably adapted to those strains, acting as asymptomatic reservoirs of leptospires in both ecological scenarios. Moreover, a high number of carriers (by PCR) and a tendency for harboring strains of serogroup Icterohaemorrhagiae could be noticed.

Despite the lack of information regarding the pathogenesis of leptospirosis in capybaras, an outstanding study succeeded on determining experimental infection with serovar Pomona (Marvulo et al., 2009). In that study the authors confirm the susceptibility of capybaras to leptospiral infection, and the absence of acute clinical signs, suggesting that animal as a chronic reservoir of leptospires.

Rattus norvegicus are well recognized as reservoirs of Icterohaemorrhagiae strains (Panti-May et al., 2016). They generally present chronic infection, with low titres and long-term bacterial shedding (Fortes-Gabriel, 2016). Although determined for Norway rats, our results suggest that those assumptions seem to be also applicable to capybaras. Unfortunately, there is scarce knowledge about the molecular and immunological mechanisms of that adaptability, and their real mechanisms remain to be elucidated.

4. Conclusions

It has been demonstrated that capybaras are massively infected by leptospires and shed those agents on the environment, both on rural and periurban regions of Western Amazon. Predominance for strains of serogroup Icterohaemorrhagiae was observed, and an important role of those animals as reservoirs of leptospires is suggested.

Conflict of interest

The authors have no conflict of interests.

Acknowledgements

The authors thank to the students of Programa de Pós Graduação em Sanidade e Produção Animal na Amazônia Ocidental (PPGESPA), to veterinarians and students of Veterinary Medicine of the Universidade Federal do Acre; Fundação de Amparo à Pesquisa do Estado do Acre (FAPAC); IBAMA. G. Martins and W. Lilenbaum are Fellows of Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). WL is a CNPq fellow.

5. References

Abreu, J.A., Krawczak, F.D., Nunes, F.P., Labruna, M.B., Pena, H.F., 2016. Anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in capybaras (*Hydrochoerus hydrochaeris*) from Itu Municipality, São Paulo. Rev. Bras. Parasitol. Vet. 25, 116-118.

Alho, C.J.R., Rondon, N.L., 1987. Habitats, population densities, and social structure of capybaras (*Hydrochaeris hydrochaeris*, Rodentia) in the Pantanal, Brazil. Rev. Bras. Zool. 4, 139–149.

Chiacchio, R.G., Prioste, F.E., Vanstreels, R.E., Knobl, T., Kolber, M., Miyashiro, S.I., Matushima, E.R., 2014. Health evaluation and survey of zoonotic pathogens in free-ranging capybaras (*Hydrochaeris hydrochaeris*). J. Wildl. Dis. 50, 496–504.

Chiebao, D.P., Valadas, S.Y., Minervino, A.H., Castro, V., Romaldini, A.H., Calhau, A.S., De Souza, R.A., Gennari, S.M., Keid, L.B., Soares, R.M., 2015. Variables associated with infections of cattle by *Brucella abortus*, *Leptospira* spp. and *Neospora* spp. in Amazon region in Brazil. Transbound. Emerg. Dis. 62, e30-36.

Cueva A.E., Rivera G., Sanchez P., Ramirez V., 2010. Incidencia de infección por *Leptospira* sp. en ronsocos (*Hydrochoerus hydrochaeris*) en cautiverio en un zoocriadero de Iquitos. Rev. Investig. Vet. Perú. 21, 106-112.

Da Silva, A.S., da Silva Krawczak, F., Soares, J.F., Klauck, V., Pazinato, R., Marcili, A., Labruna, M.B., 2016. Seroprevalence of *Trypanosoma evansi*

infection in capybaras (*Hydrochoerus hydrochaeris*) from a nonendemic area in Brazil. J. Vet. Diagn. Invest. 28, 171-174.

Deem, S.L., Emmons, L.H., 2005. Exposure of free-ranging maned wolves (*Chrysocyon brachyurus*) to infectious and parasitic disease agents in the Noël Kempff Mercado National Park, Bolivia. J. Zoo. Wildl. Med. 36, 192-197.

Donaires, L.F., Céspedes, M.J., Sihuincha, M.G., Pachas, P.E., 2012. Environmental and social determinant factors for the reemergence of leptospirosis in the Amazon region of Peru, 2012. Rev. Peru Med. Exp. Salud Publica. 29, 280-284.

Fortes-Gabriel, E., Carreira, T., Vieira, M.L., 2016. First isolates of *Leptospira* spp., from rodents captured in Angola. Am. J. Trop. Med. Hyg. *In Press*. doi:10.4269/ajtmh.15-0027.

Furtado, M.M., Gennari, S.M., Ikuta, C.Y., Jácomo, A.T., de Morais, Z.M., Pena, H.F., Porfírio, G.E., Silveira, L., Sollmann, R., de Souza, G.O., Tôrres, N.M., Ferreira Neto, J.S., 2015. Serosurvey of smooth *Brucella*, *Leptospira* spp. and *Toxoplasma gondii* in free-ranging jaguars (*Panthera onca*) and domestic animals from Brazil. PLoS One. 10, e0143816.

García-Esponda, C.M., Candela, A.M., 2016. Hindlimb musculature of the largest living rodent *Hydrochoerus hydrochaeris* (Caviomorpha): Adaptations to semiaquatic and terrestrial styles of life. J. Morphol. 277, 286-305.

Haake, D.A., Levett, P.N., 2015. Leptospirosis in humans. Curr. Top. Microbiol. Immunol. 387, 65-97.

Hamond, C., Martins, G., Loureiro, A.P., Pestana, C., Lawson-Ferreira, R., Medeiros, M.A., Lilenbaum, W. 2014. Urinary PCR as an increasingly

useful tool for an accurate diagnosis of leptospirosis in livestock. Vet. Res. Commun. 38, 81-85.

Jori, F., Galvez, H., Mendoza, P., Cespedes, M., Mayor, P., 2009. Monitoring of leptospirosis seroprevalence in a colony of captive collared peccaries (*Tayassu tajacu*) from the Peruvian Amazon. Res. Vet. Sci. 86, 383-387.

King, J.D., Congdon, E., Tosta, C., 2010. Evaluation of three immobilization combinations in the capybara (*Hydrochoerus hydrochaeris*). Zoo Biol. 29, 59-67.

Langoni, H., Kuribara, I.Y., Ferreira Lopes Correa, A.P., Ullmann, L.S., Sánchez, G.P., Lucheis, S.B., 2016. Anti-leptospirosis agglutinins in Brazilian capybaras (*Hydrochoerus hydrochaeris*). J. Venom. Anim. Toxins Incl. Trop. Dis. 27, 22-24.

Loureiro, A.P., Martins, G., Pinto, P., Narduche, L., Teixeira, R.C., Lilenbaum, W., 2015. Usage of a selective media (EMJH-STAFF) in primary culturing of pathogenic leptospires from bovine clinical samples. Lett. Appl. Microbiol. 61, 603-606.

Marvulo, M.F.V., Paula, C.D., Ferreira, P.M., Morais, Z.M., Delbem, A.C.B., Favero, A.C.M., Miraglia, F., Castro, V., Genovez, M.E., Ferraz, K., Verdade, L.M., Fellipe, P.A.N., Ferraz, E., Penteado, M., Ferreira Neto, J.S., Ferreira, F., Vasconcellos, S.A., 2002. Detection of *Leptospira* in two free living populations of capybaras (*Hydrochaeris hydrochaeris*) from São Paulo state, Brazil. Bridgetown: International Leptospirosis Society (ILS).

Marvulo, M.F., Silva, J.C., Ferreira, P.M., de Morais, Z.M., Moreno, A.M., Doto, D.S., Paixão, R., Baccaro, M.R., Vasconcellos, S.A., Ferreira Neto, J.S.,

2009. Experimental leptospirosis in capybaras (*Hydrochaeris hydrochaeris*) infected with *Leptospira interrogans* serovar Pomona. J. Zoo Wildl. Med. 40, 726–730.

Mathews, P.D., da Silva, V.M., Rosas, F.C., d'Affonseca Neto, J.A., Lazzarini, S.M., Ribeiro, D.C., Dubey, J.P., Vasconcellos, S.A., Gennari, S.M., 2012. Occurrence of antibodies to *Toxoplasma gondii* and *Leptospira* spp. in manatees (*Trichechus inunguis*) of the Brazilian Amazon. J. Zoo Wildl. Med. 43, 85-88.

Monje, L.D., Nava, S., Eberhardt, A.T., Correa, A.I., Guglielmone, A.A., Beldomenico, P.M., 2015. Molecular detection of the human pathogenic *Rickettsia* sp. strain Atlantic rainforest in *Amblyomma dubitatum* ticks from Argentina. Vector Borne Zoonotic Dis. 15, 167-169.

Monte, L.G., Jorge, S., Xavier, M.A., Leal, F.M., Amaral, M.G., Seixas, F.K., Dellagostin, O.A., Hartleben, C.P., 2013. Molecular characterization of virulent *Leptospira interrogans* serogroup Icterohaemorrhagiae isolated from *Cavia aperea*. Acta Trop. 126, 164-166.

Muñoz, D.K.; Cornejo, C., Rivera, G.H., 2014. Anticuerpos contra leptospira en capibaras (*Hydrochoerus hidrochaeris*) de un zoocriadero de la amazonía peruana. Rev. Investig. Vet. Peru. 11, 167-169.

Obiegala, A., Woll, D., Karnath, C., Silaghi, C., Schex, S., Eßbauer, S., Pfeffer, M., 2016. Prevalence and genotype allocation of pathogenic *Leptospira* species in small mammals from various habitat types in Germany. PLoS Negl. Trop. Dis. 10, e0004501.

OIE. Leptospirosis, 2014. In: Manual of diagnostic tests and vaccines for terrestrial animals. World Organization for Animal Health, Paris.

Panti-May, J.A., Carvalho-Pereira, T.S., Serrano, S., Pedra, G.G., Taylor, J., Pertile, A.C., Minter, A., Airam, V., Carvalho, M., Júnior, N.N., Rodrigues, G., Reis, M.G., Ko, A.I., Childs, J.E., Begon, M., Costa, F., 2016. A two-year ecological study of Norway rats (*Rattus norvegicus*) in a Brazilian urban slum. PLoS One. 11, e0152511.

Silva, E.F., Seyffert, N., Jouglard, S.D.D., Athanazio, D.A., Dellagostin, O.A., Brod, C.S., 2009. Seroprevalence of leptospiral infection in capybaras (*Hydrochoerus hydrochaeris*) in a slaughterhouse of Rio Grande do Sul, Brazil. Pesq. Vet. Bras. 29, 174–176.

APÊNDICE

Apêndice A – Tabela com o resultado sorológico (MAT),molecular (PCR) e cultura bacteriana de capivaras oriundas de área urbana e rural na Amazônia Ocidental, Brasil.

N	ID	Local	Sorologia – MAT		Urina - PCR	Cultura Bacteriana	Sorogrupagem
			Sorogrupo	Título			
1	521	Urbana	Negativo		Negativo	Negativo	
2	1505	Urbana	Negativo		Negativo	Negativo	
3	7648	Urbana	-		Negativo	Negativo	
4	6582	Urbana	Negativo		Positivo	Positivo	Icterohaemorrhagiae
5	5210	Urbana	Bataviae	100	Negativo	Negativo	
6	5940	Urbana	Negativo		Negativo	Positivo	Icterohaemorrhagiae
7	683	Urbana	Negativo		Negativo	Negativo	
8	772	Urbana	Negativo		Positivo	Negativo	
9	6266	Urbana	Negativo		Negativo	Negativo	
10	000	Urbana	-		Negativo	Negativo	
11	7591	Urbana	Negativo		Negativo	Negativo	
12	6510	Urbana	Pomona	200	Negativo	Negativo	
13	9088	Urbana	Pomona	100	Negativo	Negativo	
14	9490	Urbana	Shermani	100	Negativo	Negativo	
15	2157	Urbana	Negativo		Negativo	Negativo	
16	3920	Urbana	Shermani	200	**Positivo**	Negativo	
17	3874	Urbana	Negativo		Negativo	Negativo	
18	3887	Urbana	Bataviae	200	Positivo	Negativo	
19	3867	Urbana	Shermani	200	Negativo	Negativo	
20	3858	Urbana	Negativo		Positivo	Negativo	
21	3889	Urbana	Shermani	200	Negativo	Positivo	Shermani
22	Ipê	Urbana	Negativo		Positivo	Negativo	
23	8805	Urbana	Negativo		Positivo	Negativo	
24	3938	Rural	Sejroe	100	Negativo	Negativo	
25	3891	Rural	Negativo		Positivo	Negativo	
26	3876	Rural	Pomona	200	-	-	
27	3941	Rural	Pomona	200	-	-	
28	3947	Rural	**I**cterohaemorrhagiae	100	-	-	
29	6549	Rural	Negativo		Positivo	Negativo	
30	001	Rural	Negativo		Positivo	Positivo	Icterohaemorrhagiae
31	5494	Rural	Icterohaemorrhagiae	800	Negativo	Positivo	Icterohaemorrhagiae
32	8360	Rural	Negativo		Negativo	Negativo	
33	5941	Rural	Icterohaemorrhagiae	100	Positivo	Positivo	Grippotyphosa
34	3902	Rural	Icterohaemorrhagiae	100	Positivo	Positivo	Icterohaemorrhagiae
35	8040	Rural	Negativo		Negativo	Negativo	
36	3926	Rural	Negativo		Positivo	Positivo	Icterohaemorrhagiae

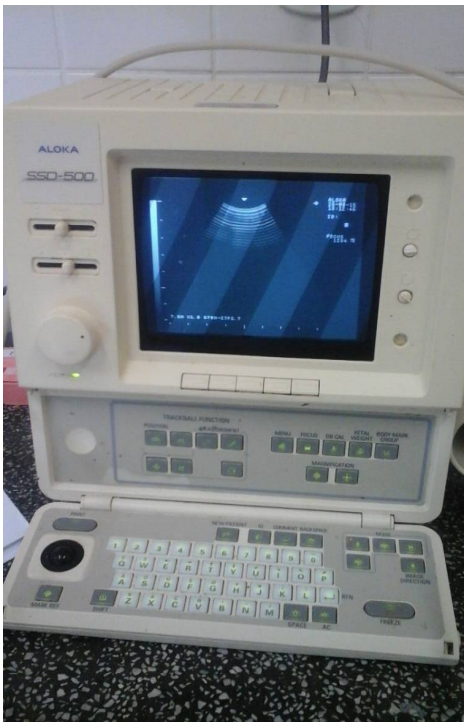
Apêndice A (cont.)

N	ID	Local	Sorologia – MAT		Urina - PCR	Cultura Bacteriana	Sorogrupagem
			Sorogrupo	Título			
37	3940	Rural	Negativo		Negativo	Negativo	
38	3861	Rural	Negativo		Negativo	Negativo	
39	3868	Rural	Sejroe	400	Negativo	Negativo	
40	3871	Rural	Negativo		Negativo	Negativo	
41	3916	Rural	Pomona	100	Negativo	Negativo	
42	3907	Rural	-		Negativo	Negativo	
43	3946	Rural	Australis	100	Negativo	Negativo	
44	3948	Rural	Negativo		Negativo	Negativo	

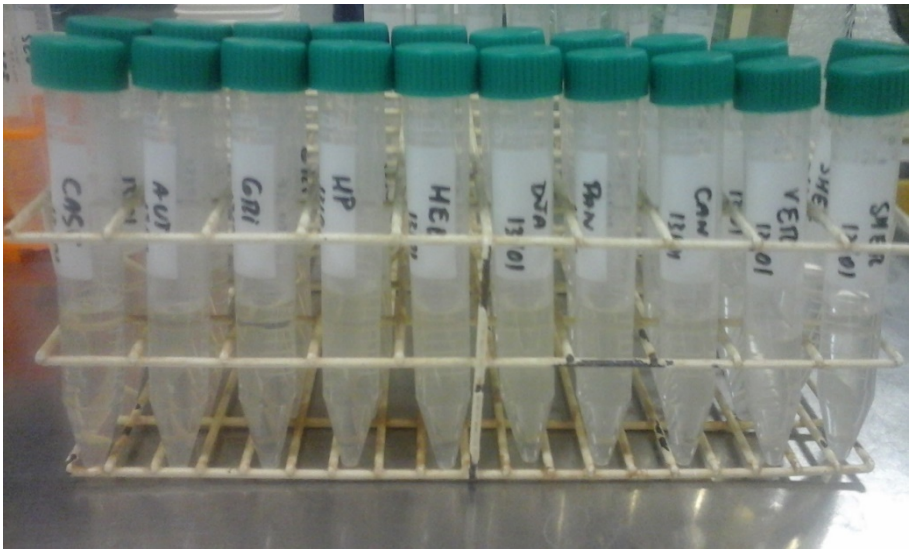
Apêndice B. Ceva com bando de capivaras capturadas em ambiente urbano.

Apêndice C. Animal sendo contido no puçá para posterior microchipagem.

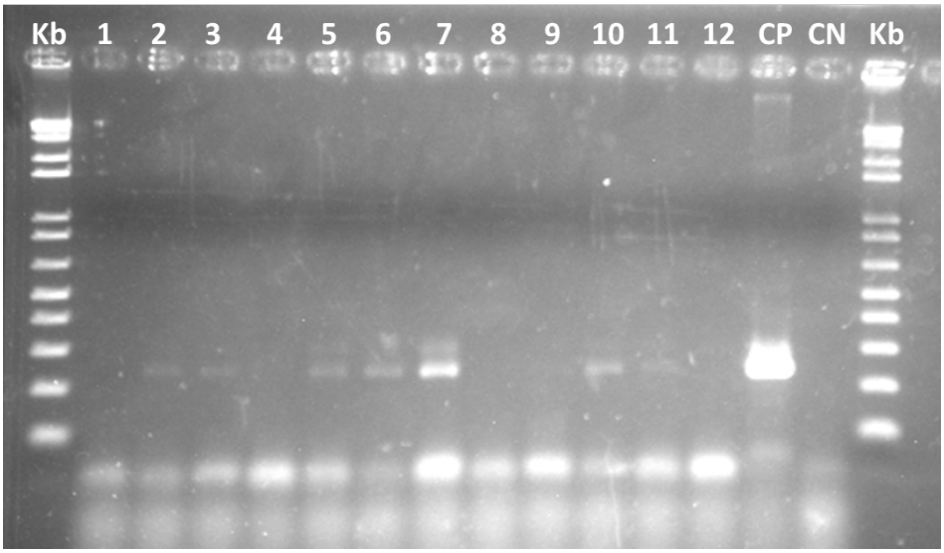
Apêndice D. Coleta de urina em capivara por meio da cistocentese com o auxílio do ultrassom.



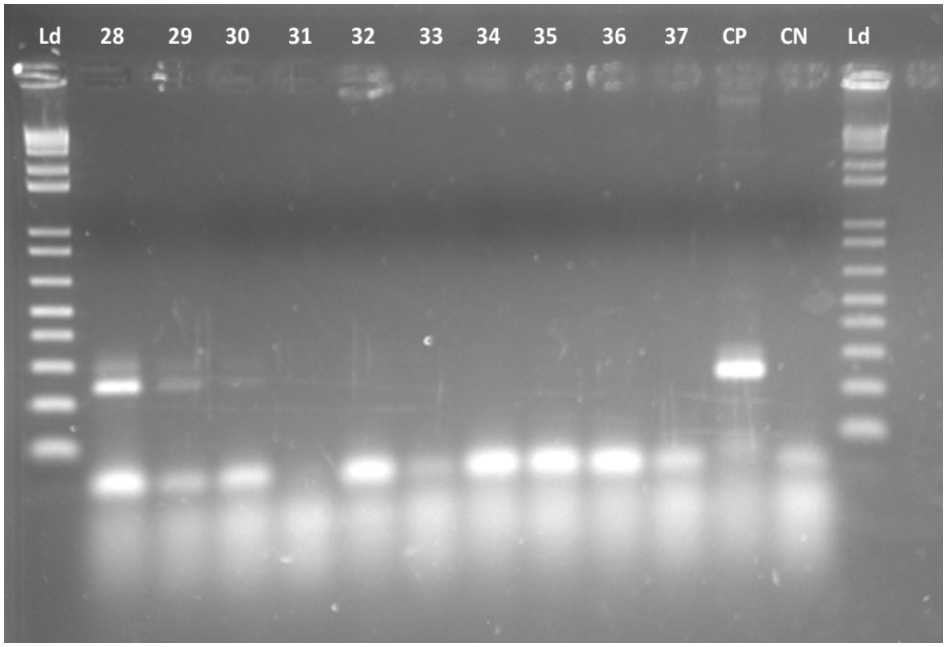
Apêndice E. Aparelho de ultrassom (Aloka, modelo SSD-500).



Apêndice F. Coleção de Leptospiras vivas mantidas em meio líquido EMJH.



apêndice G. Resultado positivo (amostras 2, 3, 5, 6, 7, 10 e 11) em gel de agarose a 2% na PCR para o gene lipL32 utilizando os primers LipL32 45F e o 286R. CP: Controle positivo; CN: Controle negativo; Kb: quilobase.



Apêndice H. Resultado positivo (amostras 28, 29 e 30) em gel de agarose a 2% na PCR para o gene lipL32 utilizando os primers LipL32 45F e o 286R. CP: Controle positivo; CN: Controle negativo; Ld: Ladder 1kb.