

Detection of *Leishmania infantum* DNA in hamsters infested with ticks collected from naturally infected dogs*

Valter dos Anjos Almeida¹, Taiane Nascimento da Hora², Nilo Fernandes Leça Júnior², Fábio Santos Carvalho³, Adriana Lopes da Silva⁴, Amauri Arias Wenceslau⁵, George Rêgo Albuquerque⁵ and Fabiana Lessa Silva^{5*}

ABSTRACT. Almeida V. dos A., da Hora T.N., Leça Júnior N.F., Carvalho F.S., da Silva A.L., Wenceslau A.A., Albuquerque G.R. & Silva F.L. **Detection of *Leishmania infantum* DNA in hamsters infested with ticks collected from naturally infected dogs.** [Detecção do DNA de *Leishmania infantum* em hamsters infestados com carrapatos coletados de cães naturalmente infectados.] *Revista Brasileira de Medicina Veterinária*, 38(4):329-333, 2016. Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, Campus Soane Nazaré de Andrade, Hospital Veterinário, Km 16, Rodovia Jorge Amado, Ilhéus, BA 45662-900, Brasil. E-mail: fabiana.lessa@gmail.com

The aim of this study was to investigate the role of *Rhipicephalus sanguineus*, the brown dog tick, in the transmission of *Leishmania infantum*. To accomplish this, we used 24 adult golden hamsters of both genders, and divided them into two groups: a control group (n = 4) and an experimental group (n = 20). The animals from the experimental group were infested with ticks obtained from dogs naturally infected with *L. infantum*. Hamsters of the control group were not infested and were maintained at the same conditions, as the infested animals. After three months of observation, animals were euthanized and they were posted to obtain samples of their blood, spleen, liver, lymph nodes, and skin. These samples were then processed by histopathology, immunohistochemistry, and polymerase chain reaction (PCR). Fourteen hamsters (70%) of the experimental group tested PCR-positive for *L. infantum* DNA in samples of buffy coat. The results of this study indicated that *R. sanguineus* ticks can transmit some forms or parts of *L. infantum* to parasitized hamsters.

KEY WORDS. *Leishmania infantum*, hamster, tick, zoonosis, public health.

RESUMO. O objetivo desse estudo foi investigar a participação de *Rhipicephalus sanguineus* na transmissão da *Leishmania infantum*. Para isso, foram utilizados 24 hamsters adultos da linhagem Golden, de ambos os sexos, os quais foram divididos em

dois grupos: controle (n = 4) e grupo experimental (n = 20). Os animais do grupo experimental foram infestados com carrapatos obtidos de cães naturalmente infectados por *L. infantum*. Os hamsters do grupo controle não foram infestados e foram man-

* Received on November 6, 2015.

Accepted for publication on March 28, 2016.

¹ Médico-veterinário, MSc. Programa de Pós-Graduação em Patologia Humana e Experimental, Centro de Pesquisas Gonçalo Moniz/Fiocruz, Rua Waldemar Falcão, 121, Candeal, Salvador, BA 40296-710. E-mail: valter_almeida@ymail.com - bolsista CAPES.

² Médico-veterinário, Programa de Pós-Graduação em Ciência Animal Departamento de Ciências Agrárias e Ambientais (DCAA), Universidade Estadual de Santa Cruz (UESC), Campus Soane Nazaré de Andrade, Hospital Veterinário, Rodovia Jorge Amado, Km 16, Ilhéus, BA 45662-900, Brasil. E-mails: taimevet@hotmail.com; niloleca@gmail.com

³ Médico-veterinário, DSc. DCAA, UESC, Campus Soane Nazaré de Andrade, Hospital Veterinário, Rodovia Jorge Amado, Km 16, Ilhéus, BA 45662-900. E-mail: fabiouesc@gmail.com

⁴ Médica-veterinária, Escola de Medicina Veterinária e Zootecnia, Universidade Federal da Bahia, Avenida Adhemar de Barros, 500, Ondina, Salvador, BA 40170-110. E-mail: drika_veterinaria@hotmail.com

⁵ Médico-veterinário, DSc, DCAA, UESC, Campus Soane Nazaré de Andrade, Hospital Veterinário, Rodovia Jorge Amado, Km 16, Ilhéus, BA 45662-900. *Author for correspondence, E-mail: fabiana.lessa@gmail.com; E-mails: wenceslau@uesc.br; gralbu@uesc.br - bolsista CNPq.

tidos sob as mesmas condições dos outros animais. Após três meses de observação, os animais foram submetidos à eutanásia e necropsia para obtenção de amostras de sangue, 35 baço, fígado, linfonodos e pele para histopatologia, imuno-histoquímica e reação da polimerase em cadeia (PCR). Quatorze hamsters (70%) do grupo experimental tiveram resultados positivos na PCR para detecção do DNA de *L. infantum* em amostras de capa de leucócitos. Os resultados obtidos nesse estudo sugerem que carrapatos *R. sanguineus* podem transmitir algumas formas ou partes de *L. infantum* para hamsters parasitados.

PALAVRAS-CHAVE. *Leishmania infantum*, hamster, carrapato, *Rhipicephalus sanguineus*, zoonose, saúde pública.

INTRODUCTION

Visceral leishmaniasis (VL) or kala-azar is a cosmopolitan zoonosis, affecting humans and domestic and wild animals. On the American continent, the disease is caused by the protozoan species, *Leishmania infantum* (= *Leishmania chagasi*) (Maurício et al. 2000). Domestic dogs (*Canis familiaris*) play a fundamental role in the maintenance of the disease in endemic regions, acting as a domestic reservoir and infection source for humans (Margonari et al. 2006, Werneck et al. 2006). Typically, transmission of *L. infantum* to mammalian hosts requires the participation of phlebotomine sand flies, which in the New World, belong to the species *Lutzomyia longipalpis* (Marcella & Gopegui 1998). However, studies have shown disease transmission in the absence of a biological vector, such as by blood transfusions (Owens et al. 2001, De Freitas et al. 2006), vertical transmission (Dubey et al. 2005), and sexual contact (Silva et al. 2009).

Ticks are vectors of a variety of important pathogens, such as blood parasites of genera *Anaplasma*, *Babesia*, *Rickettsia* and viruses (Jongejan & Uilenberg 2004). The Brown dog tick, *Rhipicephalus sanguineus*, is a vector of important pathogens with zoonotic implications (Dantas-Torres 2010b). Initial investigations regarding the hypothesis that ticks can act as vectors for parasites of the genus *Leishmania* dates to the beginning of the twentieth century. In 1930, Blanc and Caminopetros (1930) demonstrated the ability of *R. sanguineus* to become infected with *Leishmania* and to transmit the infection to rodents after inoculating them with an injection of crushed arthropods.

More recently, Coutinho et al. (2005) detected the DNA of *L. chagasi* in *R. sanguineus* removed from dogs with CVL (Canine Visceral Leishmania-

sis) through PCR analysis. These authors also infected 69 hamsters by oral and peritoneal inoculation of mashed ticks originating from infected dogs. The same results were confirmed by serological techniques, PCR, and direct examination of smears from the spleen and liver. Additionally, Dantas-Torres et al. (2010a) detected the presence of *L. infantum* kDNA in the salivary glands of *R. sanguineus* removed from naturally infected dogs. When analyzed together, these findings suggest that *R. sanguineus* may play a role in the transmission of *L. infantum* to other hosts. Despite these findings, the role of *R. sanguineus* in the transmission of CVL is still unknown. This work aimed to investigate the ability of *R. sanguineus* to transmit *L. infantum* to golden hamsters (*Mesocricetus auratus*).

MATERIAL AND METHODS

Study location

The research was conducted in the Municipality of Ilhéus, Bahia, Brazil (14°47'S; 39°02' W). At this location, previous entomological research did not detect *L. longipalpis* (Carvalho et al. 2010) and, as is currently known, there is no cases of autochthonous CVL.

Tick collection

Non-gravid *R. sanguineus* females were manually removed from symptomatic and serologically positive dogs for CVL as confirmed by the enzyme linked immunosorbent assay (ELISA) and indirect immunofluorescence reaction (IFAT) tests. The ELISA and IFAT tests were conducted at the Center for Control of Zoonosis at Belo Horizonte in the State of Minas Gerais, Brazil, an endemic region for CVL. After brown dog tick specimens were collected, they were identified morphologically and maintained in sealed containers until their arrival at the experimental location. The experimental golden hamsters were infested with these ticks within three days after being obtained.

Experimental animals

To perform this study, 24 adult Golden hamsters from both genders were used and they were provided by the UESC Breeding and Maintenance Station for Laboratory Animals. The animals maintained in mini-isolators in a controlled environment at 22° C under humane conditions and in accordance with the Ethical Principles in Animal Experimentation. Each mini-isolator accommodated two animals. Hamsters received food and water *ad libitum* during the experimental period.

This project was certified by the Committee for the Ethical Use of Experimental Animals (CEUA/UESC) by the protocol # 022/09. As an additional biosafety measure, throughout the experimental phase, the upper part of each mini-isolator was sealed with fine mesh and held in place by using double-faced, strong adhesive tape.

Experimental groups

Hamsters were divided into two groups: a control group and experimental group were consisted of 4 and 20 animals respectively. Each animal of the experimental group was infested with 3 to 5 non-engorged *R. sanguineus* adult females. The ticks were placed on the hamster's dorsal region under a plastic cover to ensure that they were not removed by the animal during the study. The number of ticks used per animal was determined by taking into consideration the amount of blood consumed per tick, such that the health hamster average weight is of 120 g, so as not to be in danger. After three days, the ticks were removed manually from the animals, and the groups of 3 to 5 ticks recovered from each hamster were placed separately into *Eppendorf* tubes and frozen at -20°C until DNA extraction. Hamsters in the control group were not exposed to ticks, but remained under the same environmental conditions as those of the experimental animals.

Clinical status, euthanasia, and necropsies

Ticks were removed and hamsters remained for three months being monitored and observed concerning the emergence of clinical signs of *L. infantum* experimental infection consisted of weight loss, alopecia and skin lesions.

At the end of the observation period, animals were sedated with an intraperitoneal injection of xylazine (10 mg/kg) and ketamine (100–200 mg/kg). Next, hamsters were anesthetized with 3% sodium pentobarbital (80–150 mg/kg, IP), and blood was sampled through cardiac puncture. Immediately thereafter, hamsters were euthanized with a lethal dose of 3% sodium pentobarbital and they were posted. During the necropsy, hamsters were evaluated for existing macroscopic changes, and samples were taken from the liver, spleen, lymph nodes, and skin for histopathological examination.

Histopathology and Immunohistochemistry

Tissue fragments were fixed in buffered 10% formalin for 24–48 h and they were processed by routine histological techniques for inclusion in paraffin. Histological slides were made from cuts at a thickness of 4–5 µm and stained with hematoxylin and eosin. The histological cuts were then evaluated under optical microscopy to determine the presence of lesions and structures compatible with amastigotes of *Leishmania*. Histological cuts on silane treated glass plates were processed for immunodetection of amastigotes following the methodology of Tafuri et al. (2004). Briefly, hyperimmune serum from hamsters naturally infected with *L. infantum* was used as a primary antibody. A detection system based on streptavidin-biotin-peroxidase (LSAB+ kit, Dako USA) was used. The marking was developed with diaminobenzidine (DAB, Dako USA). For each test was used a histological cut from either the liver, spleen, or skin from a dog naturally infected with *L. infantum* as a positive control

DNA extraction and PCR

The blood samples were centrifuged for 15 min at 3400 rpm, and then 100µL were extracted from the

buffy coat. Hamster tissue samples and groups of recovered ticks were combined and crushed in liquid nitrogen. Afterward, the peripheral blood leukocytes and 100µg of each tissue and group of ticks were processed for DNA extraction by using the Easy-DNA™ Kit (Invitrogen®) following the manufacturer's recommendations. The final DNA concentration in each sample was established by optical spectrophotometry (optical density at 260nm). To detect the presence of *Leishmania* in each DNA sample, we used the primers, RV1:5'-CTTTCTGGTCCCGCGGGTAGG-3' and RV2:5'-CCACCTGGCCTATTTTACACCA-3', to amplify the kDNA164 min circle region (Lachaud et al. 2002). To perform the PCR, we used PCR Super Mix kit (Invitrogen®) with a volume of 17 µL of SuperMix, 2 µL (0.2µM) of each primer, and 4 µL (50 ng/µL) of DNA. The thermal cycling conditions were as follows: hold for 5 min at 94°C, followed by 35 cycles of 1min at 94°C, 1 min at 59°C, 1 min at 72°C, and a final 10-min extension at 72°C (Lachaud et al. 2002). The PCR products were visualized by electrophoresis in a 2% agarose gel. DNA from a sample of *L. chagasi* (Strain MHOM/BR2000/170 Merivaldo) was used as a positive control, and ultra-pure water was used as a negative control. An ABI PRISM-3100 Genetic Analyzer (Applied Biosystems) was used to sequence the amplified PCR products. Each DNA model (45 ng) was marked with 3.2pmol of the ECAN5 primer and 2 µL of BigDye Terminator v3.1 Ready Reaction Mix (BigDye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems) in a final volume of 10 µL. The marking reactions were performed in a GeneAmpPCR System 9700 (Applied Biosystems) with an initial denaturation phase at 96°C for 3 min followed by 25 cycles at 96°C for 10 s, 55°C for 5 s, and 60°C for 4 min. The program BLAST (<http://www.ncbi.nlm.nih.gov/BLAST.cgi>) was used for comparison and analysis of the obtained sequences.

RESULTS

During the observation period, none of the hamsters presented clinical signals consistent with VL. A microscopical evaluation of the HE-stained tissues did not show pathological alterations. Structures, compatible with amastigotes of *Leishmania*, were not visualized in the histological cuts stained by the immunohistochemistry technique. The PCR performed using DNA extracted from peripheral blood leukocytes showed positive results in 70% (14/20) of the animals from the experimental group (Table 1). No PCR performed using DNA extracted from hamster tissue samples was positive for *L. infantum*. All examinations made in the control group hamsters were negative. The positive samples of peripheral blood leukocytes sequenced, and the results showed a satisfactory classification, revealing 92 and 99% similarity when compared to those stored in GenBank (Accession No's: EU370899.1; EU370893.1;203 EU370898.1).

Table 1. Detection of *Leishmania infantum* DNA in hamsters experimentally infested with *Rhipicephalus sanguineus* collected from naturally infected dogs.

Variables	Animals ^a	
	Control	Infested
Clinical signs	0/4	0/20
PCR of blood samples	0/4	14/20
Histopathological changes	0/4	0/20

^a Positive samples per group of animals

DISCUSSION

The success and progression of infection by the genus *Leishmania* in animal models depend not only on the host but also on a combination of other factors, such as the inoculated species, strain virulence, nature of the inoculating agent, amount of parasites, and form of inoculation (Moreno & Alvar 2002). Hamsters of the golden lineage are susceptible to a variety of intracellular pathogens, and therefore, constitute an excellent model for the study of a series of human infectious diseases, as they are one of the first animal models used for the study of VL (Garg & Dube 2006). This model is being widely used in research involving VL (Goto et al. 1987, Paciello et al. 2010) because the clinical and pathological characteristics of the disease in these animals are similar to those in humans (Melby et al. 2001). The role of *R. sanguineus* in the transmission of *Leishmania* is still unknown to the scientific community. Recently, some studies have attempted to clarify the role of these ticks in the epidemiology of canine VL (Coutinho et al. 2005, Dantas-Torres et al. 2010a, Dantas-Torres et al. 2011, Campos & Costa 2014), and suggest that *Leishmania* parasites may remain alive within ticks even after the nymph to adult molt (Colombo et al. 2011). Recently, for the first time, the presence of kDNA of *L. infantum* was detected in the salivary glands of *R. sanguineus* removed from dogs naturally infected with *Leishmania*, by using the PCR technique (Dantas-Torres et al. 2011). During feeding by *R. sanguineus*, the ticks inoculate the host with a huge quantity of substances from their salivary glands (Dantas-Torres 2008). The results of the present study show the presence of *L. infantum* DNA in the blood of 14 of the 20 hamsters born and bred in confinement and infested with ticks positive for *L. infantum*. The similarity between the analyzed sequences, when compared to those in GenBank, varied between 92 and 99%. Homology values lower than 99% may be because of sequencing errors since the PCR products were not purified. These results corroborate with those from PCR amplification, confirming the parasite's presence in the blood of the animals.

Our results demonstrate the possibility that the dog brown ticks *R. sanguineus* can transmit *L. infantum* to animals; however, we did not directly observe the parasite in the host and there were no clinical signs consistent with the disease in the hamsters studied. In addition, at present, there is no method to determine whether transmission of *L. infantum* occurred or the presence was because of the inoculated fragments of the parasite or dead parasites. Although it has been confirmed that the most probable mode of transmission of VL is by *L. longipalpis*, alternative forms of transmission may have a relevant role in the dissemination of this disease and should be considered in the development of control strategies. In this particular case, it is believed that the probable transmission of *L. infantum* by *R. sanguineus* may have widespread epidemiological implications since these ticks are widely distributed due to their ability to adapt and also due to the cosmopolitan distribution of their host, the domestic dog (Dantas-Torres 2010b). In the present study, we detected *L. infantum* DNA in 6 of 13 (46%) of the recovered groups of ticks. Dantas-Torres et al. (2010a) detected *L. infantum* DNA in 12.3% of the ticks analyzed, while Coutinho et al. (2005) found the same in 15.4% of the arthropods evaluated. The percentages of infection are high, especially when one considers the infection rates in phlebotomines in endemic areas, which are usually lower, ranging between 0.25% and 3.9% (Paiva et al. 2006, Soares et al. 2010, Michalsky et al. 2011). Despite the fact that *R. sanguineus* are species-specific (for dogs), there are countless reports in the literature of tick parasitism in humans (Dantas-Torres et al. 2006, Louly et al. 2006). Dantas-Torres (2010b) reports that when exposed to high temperatures, these arthropods attach to and feed faster on humans. These observations suggest that, if the hypothesis of vectorial competence is confirmed, *R. sanguineus* may also have importance in the transmission of VL to humans.

If the vectorial competence of *R. sanguineus* in the transmission of *L. infantum* is verified, it will have a substantial impact on strategies for the control of this disease. Besides the traditionally recommended measures, such as control of the main phlebotomine vector and elimination of infected dogs, the control of ticks may also become an important strategy in the control of canine and human VL. The inspection and elimination of ticks on dogs will limit the introduction of *L. infantum* into disease free areas.

CONCLUSIONS

In conclusion, our results demonstrate that *R. sanguineus* can transmit some forms or parts of *L. infantum*; however, further studies are needed to determine whether these forms are capable of inducing VL and whether the cycle of the parasite remains continuous in newly infected hosts. Confirmation of this will have a huge impact on the epidemiology of VL with implications for the strategies employed in controlling the disease.

Acknowledgments. This study was supported by the “Fundação de Amparo à Pesquisa do Estado da Bahia” (FAPESB, Salvador, Bahia) and the “Universidade Estadual de Santa Cruz” (UESC, Ilhéus, Bahia). VAA had a scholarship from CNPq. We are thankful to the “Centro de Controle de Zoonoses - Prefeitura Municipal de Belo Horizonte” for providing the ticks.

REFERENCES

- Blanc G. & Caminopetros J. La transmission du Kala-Azar méditerranéen en par une tique: *Rhipicephalus sanguineus*. *Comptes Rendus de l'Académie des Sciences*, 191:1162-1164, 1930.
- Campos J.H.F. & Costa F.A.L. Participação do *Rhipicephalus sanguineus* no ciclo infeccioso da leishmaniose visceral canina em Teresina, Piauí, Brasil. *Revista do Instituto de Medicina Tropical de São Paulo*, 56:297-300, 2014.
- Carvalho S.M.S., dos Santos P.R.B., Lanza H. & Brandão-Filho S.P. Diversidade de flebotomíneos no Município de Ilhéus, Bahia. *Epidemiologia e Serviços de Saúde*, 19:239-244, 2010.
- Colombo F.A., Odorizzi R.M.F.N., Laurenti M.D., Galati E.A.B., Canavez F. & Pereira-Chioccola V.L. Detection of *Leishmania (Leishmania) infantum* RNA in fleas and ticks collected from naturally infected dogs. *Parasitology Research*, 109:267-274, 2011.
- Coutinho M.T.Z., Bueno L.B., Sterzik A., Fujiwara R.T., Botelho J.R., de Maria M., Genaro O. & Linardi, P.M. Participation of *Rhipicephalus sanguineus* (Acari: Ixodidae) in the epidemiology of canine visceral leishmaniasis. *Veterinary Parasitology*, 128:149-155, 2005.
- Dantas-Torres F. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. *Veterinary Parasitology*, 152:173-185, 2008.
- Dantas-Torres F. Ticks as vectors of *Leishmania* parasites. *Trends in Parasitology*, 27:155-159, 2011.
- Dantas-Torres F., Figueiredo L.A. & Brandão-Filho S.P. *Rhipicephalus sanguineus* (Acari:Ixodidae), the brown dog tick, parasitizing humans in Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 39:64-67, 2006.
- Dantas-Torres F., Lorusso V., Testini G., De Paiva-Cavalcanti M., Figueiredo L.A., Stanneck D., Menck N., Brandão-Filho S.P., Alves L.C. & Otranto D. Detection of *Leishmania infantum* in *Rhipicephalus sanguineus* ticks from Brazil and Italy. *Parasitology Research*, 106:857-860, 2010a.
- Dantas-Torres F., Martins T.F., De Paiva-Cavalcanti M., Figueiredo L.A., Lima B.S. & Brandão-Filho S.P. Transovarial passage of *Leishmania infantum* kDNA in artificially infected *Rhipicephalus sanguineus*. *Experimental Parasitology*, 125:184-185, 2010b.
- De Freitas E., Melo M.N., Da Costa-Val A.P. & Michalick M.S. Transmission of *Leishmania infantum* via blood transfusion in dogs: potential for infection and importance of clinical factors. *Veterinary Parasitology*, 137:159-167, 2006.
- Dubey J.P., Rosypal A.C., Pierce V., Scheinberg S.N. & Lindsay D.S. Placentitis associated with leishmaniasis in a dog. *Journal of the American Veterinary Medical Association*, 227:1266-1269, 2005.
- Garg R. & Dube A. Animal models for vaccine studies for visceral leishmaniasis. *Indian Journal of Medical Research*, 123:439-459, 2006.
- Goto H., Carnero L.R., Ribeiro M.V.L., Hatsumi Y.E. & Seixas D.M.I. Immunological parameters of visceral leishmaniasis of *Leishmania major*-infected golden hamsters. *Allergologia et Immunopathologia*, 15:349-353, 1987.
- Jongejan F. & Uilenberg G. The global importance of ticks. *Parasitology*, 129:S3-S14, 2004.
- Lachaud L., Marchergui-Hammami S., Chabbert E., Dereure J., Dedet J.P. & Bastien P. Comparison of six PCR methods using peripheral blood for detection of canine visceral leishmaniasis. *Journal of Clinical Microbiology*, 40:210-215, 2002.
- Louly C.C.B., Fonseca I.N., Oliveira V.F. & Borges L.M.F. Ocorrência de *Rhipicephalus sanguineus* em trabalhadores de clínicas veterinárias e canis, no município de Goiânia, GO. *Ciência Animal Brasileira*, 7:103-106, 2006.
- Margonari C., Freitas C.R., Ribeiro R.C., Moura A.C., Timbó M., Gripp A.H., Pessanha J.E. & Dias E.S. Epidemiology of visceral leishmaniasis through spatial analysis, in Belo Horizonte municipality, state of Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz*, 101:31-38, 2006.
- Marsella R. & Gopegui R.R. Leishmaniasis: a re-emerging zoonosis. *International Journal of Dermatology*, 37:801-814, 1998.
- Maurício I.L., Stothard J.R. & Miles M.A. The strange case of *Leishmania chagasi*. *Parasitology Today*, 16:188-199, 2000.
- Melby P.C., Chandrasekar B., Zhao W. & Coe J.E. The hamster as a model of human visceral leishmaniasis: progressive disease and impaired generation of nitric oxide in the face of a prominent Th1-like cytokine response. *Journal of Immunology*, 166:1912-1920, 2001.
- Michalsky E.M., Guedes K.D.E.S., Lara E.S.O., França-Silva J.C., Dias C.L. & Barata R.A. Natural infection with *Leishmania infantum chagasi* in *Lutzomyia (Lutzomyia) longipalpis* (Diptera: Psychodidae) sandflies captured in the municipality of Janaúba, State of Minas Gerais, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 44:58-62, 2011.
- Moreno J. & Alvar J. Canine leishmaniasis: epidemiological risk and the experimental model. *Trends in Parasitology*, 18:399-405, 2002.
- Owens S.D., Oakley D.A., Marrayott K., Hatchett W., Walton R., Nolan T.J., Newton A., Steurer F., Schantz P. & Giger U. Transmission of visceral leishmaniasis through blood transfusions from infected English foxhounds to anemic dogs. *Journal of the American Veterinary Medical Association*, 219:1076-83, 2001.
- Paiva B.R., Secundino N.F., Nascimento J.C., Pimenta A.P.F., Galati E.A., Junior H.F. & Malafronte S. Detection and identification of *Leishmania* species in field-captured phlebotomine sandflies based on mini-exon gene PCR. *Acta Tropica*, 99:252-259, 2006.
- Paciello O., Wojcik S., Gradoni L., Oliva G., Trapani F., Iovane V., Politano L. & Papparella S. Syrian hamster infected with *Leishmania infantum*: a new experimental model for inflammatory myopathies. *Muscle & Nerve*, 41:355-361, 2010.
- Silva F.L., Oliveira R.G., Silva T.M., Xavier M.N., Nascimento E.F., Santos R.L. Venereal transmission of canine visceral leishmaniasis. *Veterinary Parasitology*, 160:55-59, 2009.
- Soares M.R.A., Carvalho C.C., Silva L.A., Lima M.S.C.S., Barral A.M.P., Rebêlo J.M.M. & Pereira S.R.F. Análise molecular da infecção natural de *Lutzomyia longipalpis* em área endêmica de leishmaniose visceral no Brasil. *Cadernos de Saúde Pública*, 26:2409-2413, 2010.
- Tafuri W.L., Santos R.L., Arantes R.M.E., Gonçalves R., Melo M.N., Michalick M.S.M. & Tafui W.L. An alternative immune histochemical method for detecting *Leishmania* amastigotes in paraffin-embedded canine tissues. *Journal of Immunological Methods*, 292:17-23, 2004.
- Werneck G.L., Costa C.H., Walker A.M., David J.R., Wand M. & Maguire J.H. Multilevel modeling of the incidence of visceral leishmaniasis in Teresina, Brazil. *Epidemiology and Infection*, 135:195-201, 2006.