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Survey of *Leishmania Infantum Chagasi* in Wild and Domestic Animals in Urban Area and Atlantic Rainforest Fragment in Northeast, Brazil

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Abstract

Objective: Survey of *Leishmania infantum chagasi* in domestic and wild mammals in urban area and a Biological Reserve in Natal, Rio Grande do Norte, Brazil.

Methods: Domestic and wild mammals were captured in Brazilian northeast Atlantic Rainforest, Rio Grande do Norte state. Serological and parasitological studies were conducted; *Leishmania* isolates were positioned in phylogeny based on small subunit rDNA (SSU rDNA) and glycosomal-3-phosphate dehydrogenase (gGAPDH) gene sequences.

Results: Blood samples were collected from 138 wild and domestic mammals, comprising 66 dogs, 52 cats and 20 marsupials. Antibodies were found in 27 dogs (40.91%), two cats (3.85%) and one *Didelphis albiventris* (5%). The cultures of popliteal lymph node aspirates were positive in nine dogs (13.64%) and from seven (10.61%) the parasite was isolated and cryopreserved. All isolates were positioned in phylogeny based on SSU rDNA and gGAPDH in the same branch with *L. infantum chagasi*.

Conclusions: The proximity of the forest fragment with humans and their domestic animals provide interference in the health of wild animals. Measures to control the population of feral cats and environmental conservation should be implemented due the importance of visceral leishmaniasis.

Keywords: *Leishmania infantum chagasi*; Serology; IFAT; Phylogeny; Domestic animals; Wild mammals

Introduction

Visceral leishmaniasis (VL) is the most severe disease caused by *Leishmania* organisms, and it is a serious public health problem [1]. The species causing visceral leishmaniasis belongs to the *L. donovani* complex of the subgenus *L. (Leishmania*) including: *L.(L.) donovani*, which causes anthroponotic visceral leishmaniasis in India, Bangladesh, Nepal and Pakistan; *L.(L.) infantum*, responsible for zoonotic leishmaniasis in the Mediterranean region (Europe and Africa); and *L.(L.) chagasi*, which shows high genetic similarity with *L. (L.) infantum*, and has been correlated with zoonotic leishmaniasis in different countries of the Americas (New World) [2].

Although the domestic dog is the most important vertebrate host and the main reservoir for human VL caused by Leishmania zoonotic species [3], the finding of positive cats for VL [4,5] suggests that felids may also have some role in the epidemiology of visceral leishmaniasis.

Furthermore, VL has been documented in several mammalian species as canids [6-10], rodents [9,11], opossums of the genus *Didelphis* [9,12] and bats [13]. The identification of infection in wildlife has stimulated debate about a sylvatic *L. infantum chagasi* transmission cycle operating independently or interacting with a domestic cycle maintained by dogs [14]. Through the identification and knowledge of the biology of natural reservoirs, more efficient measures can be taken for prevention and control of leishmanioses.

In Brazil, *Lutzomyia longipalpis* and *Lutzomyia cruzi* are the sand fly species described as main vectors of *L. infantum chagasi* [9,15]. The Brazilian Northeast is a geopolitical region of great concern to present the favorable weather conditions in the proliferation of mosquitoes and disease transmission, as a long coastline that features high temperatures and humid climate [16].

In this study, we conducted a survey of Leishmania infection among small mammals and domestic animals in an urban area and from a Biological Reserve, located in the municipality of Natal, State of Rio Grande do Norte, throught serological, parasitological, and in vitro isolation of Leishmania organisms, which were genetically characterized by phylogenetic analysis relationships based on SSU rDNA and gGAPDH genes.

Materials and Methods

Study area and blood samples

The biological Reserve "Parque da Cidade Dom Nivaldo Monte" (64 ha), located in Natal municipality (5° 51' 3.63"S, 35° 13' 39.75"W) ,

Rio Grande do Norte state and is a remnant of Atlantic Rainforest surrounded by dune formations and resting (tropical moist broadleaf forest). The local climate is tropical humid with annual temperature of 26°C and annual rainfall of around 2500 mm, with a rainy season from March to July. The Biological Reserve promotes the integration of two neighborhoods areas with environmental and socioeconomic distinct characteristics and the northern portion of Biological Reserve is surrounded by clandestine occupations. One of the main problems of this Reserve is the presence of a high number of abandoned domestic cats.

Regarding to the wild animals, Pittfall and Sherman traps were used for capture of small mammals in two campaigns in dry (October 2012) and rainy (February 2013) seasons. The small mammals and were caught were anesthetized (ketamine and xylazine), and blood samples were collected through heart puncture. Animals were identified using identification keys and original descriptions [17]. Some animal specimens that could not be identified at species level during the field collection were euthanized, fixed and deposited in Zoology department of Pontifícia Universidade Católica de Minas Gerais. Domestic cats present in the area were captured and blood samples were collected.

In parallel to our field work, blood samples were collected from dogs during field campaigns handled by the Zoonoses Control Center (CCZ) of the municipality of Natal. All procedures with animals in the present study were previously approved by the Animal Research Committee of the Faculty of Veterinary Medicine of University of São Paulo, and by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA).

Isolation of *Leishmania*

For isolation of *Leishmania* organisms, blood samples were collected from wild and domestic animals, and popliteal lymph node aspirates were obtained through fine-needle puncture from the dogs and cats. The aspirates contents were inoculated into Vacutainer tubes containing a biphasic medium consisting of 15% sheep red blood cells as the solid phase (blood agar base), overlain by liquid LIT medium supplemented with 20% Fetal Bovine Serum [18].The culture was incubated at 28°C and grown in LIT medium for DNA preparation.The isolates were cryopreserved in liquid nitrogen in the Brazilian Trypanosomatid Collection (Coleção Brasileira de Tripanossomatídeos, CBT), in the Department of Preventive Veterinary Medicine and Animal Health, School of Veterinary Medicine, University of São Paulo, Brazil.

Serological diagnosis of *Leishmania infantum chagasi*

Anti-*Leishmania infantum chagasi* antibodies were detected by indirect fluorescent antibody test (IFAT), with a cutoff value of 1:40. Promastigote forms of the *L. infantum chagasi* (CBT 96 strain) were used as the antigen, as described by Ferrer et al. [19]. *L. infantum chagasi* IFAT-positive sera were tested at twofold serial dilutions until the last positive dilution, and rabbit anti-canine IgG conjugate (Sigma, USA) was used in both tests. Positive and negative control sera were added to each slide.

Molecular and phylogenetic analysis

The DNA from *Leishmania* cultures was extracted using the phenol-chloroform method. DNA samples were subjected to the conventional polymerase chain reaction (PCR) for trypanosome barcoding on a fragment of approximately 900 base pairs (bp) of the V7V8 SSU rDNA [18,20] and gGAPDH gene [21]. PCR products of the expected size were purified and sequenced in an automated sequencer (ABI Prism 310). The nucleotide sequences generated were deposited in GenBank (Table 1).

Trypanosomatid Species	CBTa	Isolate codeb	Host	Geographic originc		Acession numberd Sequences	
						SSUrRNA	gGAPDH
L. donovani	77	MHOM/ET/1967/ L82;HV3;LV9	*Homo sapiens*	Ethiopia	ET	KF041801	FR799617
L. donovani	89	MHOM/ET/ 1967/HU3	*Homo sapiens*	Ethiopia	ET	KF041800	XM_003862962
L. infantum		JCPM5		Europe		M81430	FR796462/ XM_001467109
L. infantum				Europe		M81429	
L. infantum chagasi	39		*Canis lupus familiaris*	Pará	BR	KF041788	KF041818
L. infantum chagasi	40		*Canis lupus familiaris*	Pará	BR	KF041789	KF041819
L. infantum chagasi	43		*Canis lupus familiaris*	Pará	BR	KF041790	KF041820
L. infantum chagasi	105		*Canis lupus familiaris*	Rio Grande do Norte	BR	KJ697707	KJ697700
L. infantum chagasi	106		*Canis lupus familiaris*	Rio Grande do Norte	BR	KJ697708	KJ697701
L. infantum chagasi	107		*Canis lupus familiaris*	Rio Grande do Norte	BR	KJ697709	KJ697702
L. infantum chagasi	108		*Canis lupus familiaris*	Rio Grande do Norte	BR	KJ697710	KJ697703

L. infantum chagasi	124		*Canis lupus familiaris*	Rio Grande do Norte	BR	KJ697711	KJ697704
L. infantum chagasi	125		*Canis lupus familiaris*	Rio Grande do Norte	BR	KJ697712	KJ697705
L. infantum chagasi	126		*Canis lupus familiaris*	Rio Grande do Norte	BR	KJ697713	KJ697706

Table 1: Trypanosomatid isolates, host, geographical origin and sequences of SSU rDNA and gGAPDH genes employed in the phylogenetic analyses performed in this study.

The sequences obtained in this study were aligned with sequences previously determined and available in GenBank (Table 2) using ClustalX [22] and were adjusted manually using GeneDoc [23]. The phylogenetic tree were constructed using maximum parsimony, as implemented in PAUP version 4.0b10 [24] with 500 bootstrap replicates. Bayesian analysis was performed using MrBayes v3.1.2 [25] with 1,000,000 replicates. Trees were sampled every 100 generations using chains, and 25% of the trees were discarded as burn-in, and the remaining trees were used to calculate Bayesian posterior probability. The GTR+I+G substituition model were used.

Animals examined	Antibody titer	Positive samples (%)	Parasitological
Dogs			
	negative	39/66 (59)	1/66 (1.5)
	40	15/66 (22)	4/66 (6.5)
	80	6/66 (9.5)	2/66 (3)
	160	4/66 (6.5)	1/66 (1.5)
	320	1/66 (1.5)	1/66 (1.5)
	640	1/66 (1.5)	
Cats			
	negative	46/48	-
	40	1/48 (2.1)	
	80	1/48 (2.1)	
Marsupials			
	negative	19/20	-
	320	1/20 (5)	

Table 2: Sero-prevalence of *Leishmania chagasi* infection by ifat in wild animals, dogs and cat from the Urban area and Atlantic Rainforest fragment in northeast, Brazil.

Results

Blood samples were collected from 138 wild and domestic mammals, comprising 66 dogs, 52 cats, and 20 marsupials (16 *Didelphis albiventris* and 4 *Monodelphis domestica*). Among the canine sera tested by IFAT, 27 (40.91%) were serologically positive with the following endpoint titers: 40 (55.55%); 80 (22.22%); 160 (14.83%) and 320 (7.4%). Among cats, only two (3.85%) were considered serologically reactive to *L. infantum chagasi*, with endpoint titers ranging from 40 to 80. Only one opossum, *Didelphis albiventris* (5%), was seroreactive with the 320 endpoint titer.

Cultures that were inoculated with canine popliteal lymph node aspirates were positive to nine dogs (13.64%); seven (10.61%) isolates were established and cryopreserved. Cultures inoculated with feline and wild animals samples were all negative.

Phylogenetic position based on concatenated V7V8 SSUrDNA and gGAPDH genes segregated the *L. donovani* complex species and all seven dogs isolates were in a single cluster (91% bootstrap/100% posterior probability and 100% of similarity) in the same branch with *L. infantum chagasi* from Brazil (Figure 1).

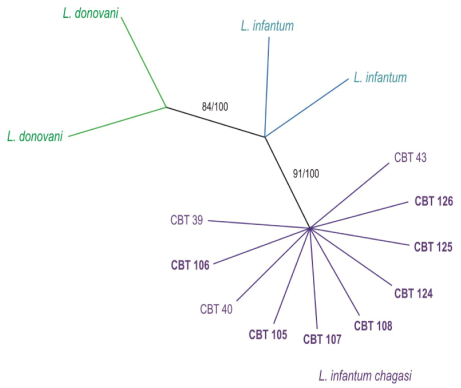

Figure 1: Dendogram based on concatenated SSU rDNA and gGAPDH sequences on 14 *Leishmania* species of the *L. donovani* complex, which was used for maximum parsimony and Bayesian inferences with 1460 characters. Numbers at nodes are the support values for the major branches (bootstrap/posterior probability; 500 replicates). The sequences obtained in this study are in bold.

Discussion

Visceral *leishmaniasis* occurs in most countries of Latin America, where nearly 90% of the cases in Brazil, especially in Northeast region of the country. This region is of great concern for presenting favorable weather conditions for the proliferation of vectors. The most populated areas are located in the coastal region with humid climate and high temperatures favoring the vector species, *Lutzomyia longipalpis* and *Lutzomyia cruzi*.

Visceral *leishmaniasis* in the Americas is caused by the species *L. infantum chagasi* that is phylogenetically distinct from other species of the *L. donovani* complex [18]. Recent study showed that the V7V8 SSU rDNA region segregated all species of the genus *Leishmania* and can be used for barcode as in other genera of trypanosomatids. In Brazil, others species of *Leishmania* was able to cause visceral

pathologies such as *L. braziliensis*. The identification or differentiation of circulating species in the area is important to take preventive measures and control of these parasites.The isolates in this study were placed in phylogenies and taxonomically confirmed as *L. infantum chagasi*.

Entomological surveys conducted in the metropolitan region of Natal demonstrated the predominance of *L. longipalpis* in urban and peridomestic region [26]. Domestic dogs are an important source of infection for vector in an urban environment and are usually associated with the occurrence of human cases [27,28].

The presence of high rates of seropositivity in dogs found in this study highlights the epidemiological scenario of the study area, indicating high exposure of dogs to *Leishmania*-infected sand flies. This scenario could be related to the expanding urban environment, which could results in higher exposure of humans to visceral *leishmaniasis* in such region [12].

Usually, cats are naturally infected by the same *Leishmania* species that affect dogs and humans in tropical and subtropical areas worldwide [29]. In the present study, 3,85% of the sampled cats were seroreactive for *Leishmania infantum chagasi*, corroborating previous reports in other endemic regions of Brazil [30,31].

The serologic response of *Leishmania*-infected cats is usually considered less specific than it is in dogs, probably by the lack of a standardized IFAT for serological evaluation of anti-*Leishmania spp*. Antibodies in cats; there is no universally accepted antibody titer cut-off value that corresponds to active infection. Cut-off titers validated for dogs are frequently applied for cats, but the immune response could be different among cats and dogs. This context, there might have frequent failures to diagnose feline *leishmaniasis* [32]. Como melhorar o diagnostic em gatos.

Unrestrained domestic cats are usually vagile, as they can actively hunter in forests and could act as a link between domestic and wild environment, disseminating the parasite because cats can be bitten by the local vector (*L. longipalpis*), as corroborated by numerous reports of skin lesions with *Leishmania-amastigotes* forms presence [4,33,34].

The search for *leishmaniasis* in opossum is justified by reports of natural infection of this animal in different regions of Brazil [12,35,36]. Previous studies showed that the presence of opossum (*Didelphis marsupialis*) was an important risk factor for the transmission of l*eishmaniasis*, since opossum populations usually have high infection rates in urban endemic area, suggesting that this species may act as a natural link between domestic and sylvatic *Leishmania* life cycles, especially because opossums and dogs can inhabit the same environment [37,38]. Moreover, opossum infection by *L. infantum chagasi* has been associated with human VL in some areas [36].

The presence of opossums serologically reactive to *L. infantum chagasi* in the present study, suggests that the opossum population of the Biological Reserve may act as a reservoir for *Leishmania* parasites, thereby corroborating the hypothesis that *D. albiventris* is one of the links of domestic and sylvatic transmission.

The Parque da Cidade Dom Nivaldo Monte Biological Reserve has a large population of stray cats and wild animals, especially opossums, which are inserted in the epidemiological chain of visceral leishmaniasis. The close relationship between pets and wild animals can, in addition, providing increased competition for wild and anthropogenic habitats, resulting in transmission of diseases from one group to the other [39].

The proximity of the forest fragment to humans and domestic animal populations may have important one-health implications, with relevance sanitary consequences for either domestic and wild animals, and humans. Because the study area is a public park frequently visited by humans, mainly tourists, measures to control the population of cats and environmental conservation measures should be implemented because of the importance of visceral *leishmaniasis*.

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References

1. Postigo JA (2010) Leishmaniasis in the World Health Organization Eastern Mediterranean Region. Int. J. Antimicrob. Agents 36: 62-65.
2. Lukes J, Mauricio IL, Schönian G, Dujardin JC, Soteriadou K, et al. (2007) Evolutionary and geographical history of the Leishmania donovani complex with a revision of current taxonomy. Proc Natl Acad Sci U S A 104: 9375-9380.
3. Souza TD, Turchetti AP2, Fujiwara RT3, Paixão TA3, Santos RL4 (2014) Visceral leishmaniasis in zoo and wildlife. Vet Parasitol 200: 233-241.
4. da Silva AV, de Souza Cândido CD, de Pita Pereira D, Brazil RP, Carreira JC (2008) The first record of American visceral leishmaniasis in domestic cats from Rio de Janeiro, Brazil. Acta Trop 105: 92-94.
5. Braga AR, Langoni H, Lucheis SB1 (2014) Evaluation of canine and feline leishmaniasis by the association of blood culture, immunofluorescent antibody test and polymerase chain reaction. J Venom Anim Toxins Incl Trop Dis 20: 5.
6. DEANE LM, DEANE MP (1954) Isolation of leishmaniae in the viscera and the skin of a fox in the kala-azar endemic zone in Sobral, Ceara]. Hospital (Rio J) 45: 419-421.
7. Deane LM, Deane MP (1955) Observações preliminares sobre a importância comparativa do homem, do cão e da raposa (Lycalopex vetulus) como reservatório de Leishmania donovani em área endêmica de Calazar no Ceará. Hospital 48: 61-76.
8. Courtenay O, Quinnell RJ, Garcez LM, Dye C (2002) Low infectiousness of a wildlife host of Leishmania infantum: the crab-eating fox is not important for transmission. Parasitology 125: 407-414.
9. Lainson R, Rangel EF (2005) Lutzomyia longipalpis and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. Mem Inst Oswaldo Cruz 100: 811-827.
10. Del Río L, Chitimia L, Cubas A, Victoriano I, De la Rúa P, et al. (2014) Evidence for widespread Leishmania infantum infection among wild carnivores in L. infantum periendemic northern Spain. Prev Vet Medicine 113: 430–435.
11. Helhazar M, Leitão J, Duarte A, Tavares L, da Fonseca IP (2013) Natural infection of synathropic rodent species Mus musculus and Rattus norvegicus by Leishmania infantum in Sesimbra and Sintra--Portugal. Parasit Vectors 6: 88.
12. Lima BS, Dantas-Torres F, Carvalho MR, Marinho-Junior JF, Almeida EL, et al. (2013) Small mammals as hosts of Leishmania spp. in a highly endemic area for zoonotic leishmaniasis in north-eastern Brazil. Trans R Soc Trop Med Hyg 107: 592-597.
13. De Lima H, Rodríguez N, Barrios MA, Avila A, Cañizales I, et al. (2008) Isolation and molecular identification of Leishmania chagasi from a bat (Carollia perspicillata) in northeastern Venezuela. Mem Inst Oswaldo Cruz 103: 412-414.

14. Quinnell RJ, Courtenay O (2009) Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. Parasitology 136: 1915-1934.
15. dos Santos SO, Arias J, Ribeiro AA, de Paiva Hoffmann M, de Freitas RA, et al. (1998) Incrimination of Lutzomyia cruzi as a vector of American visceral leishmaniasis. Med Vet Entomol 12: 315-317.
16. Ximenes MF, Silva VP, Queiroz PV, Rego MM, Cortez AM, et al. (2007) Phlebotomine (Diptera: Psychodidae) and leishmaniasis in Rio Grande do Norte State Brazil: anthropic environment responses. Neotrop Entomol 36: 128-37.
17. Vizotto LD, Taddei VA (1973) Chave para a determinação de quirópteros brasileiros. São José do Rio Preto, Francal: 72.
18. Marcili A, Sperança MA, Costa AP, Madeira MF, Soares HS, et al. (2014) Phylogenetic relantionships of Leishmania species based on trypanosomatid barcode (SSU rDNA) and gGAPDH genes: taxonomic revision of Leishmania (L.) infantum chagasi in South America. Infect Genet Evol in press.
19. Ferrer L, Aisa MJ, Roura X, Portús M (1995) Serological diagnosis and treatment of canine leishmaniasis. Vet Rec 136: 514-516.
20. Marcili A, Lima L, Cavazzana M, Junqueira AC, Veludo HH, et al. (2009) A new genotype of Trypanosoma cruzi associated with bats evidenced by phylogenetic analyses using SSU rDNA, cytochrome b and Histone H2B genes and genotyping based on ITS1 rDNA. Parasitology 136: 641-655.
21. Hamilton PB, Stevens JR, Gaunt MW, Gidley J, Gibson WC (2004) Trypanosomes are monophyletic: evidence from genes for glyceraldehyde phosphate dehydrogenase and small subunit ribosomal RNA. Int J Parasitol 34: 1393-1404.
22. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876–4882.
23. Nicholas KB, Nicholas HB jr, Deerfield DW (1997) GeneDoc: Analysis and Visualization of Genetic Variation. EMBNEW NEWS 4:14.
24. Swofford D L (2002) PAUP*: Phylogenetic Analysis using Parsimony (*and Other Methods), Beta Version 4.0b10. Sinauer and Associates, Sunderland, Massachusetts, USA.
25. Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754-755.
26. Cortez AM, Silva VP, Queiroz PV, Andrade HT, Loiola MI, et al. (2007) Vertical stratification and development aspects of phlebotomine sand flies (Diptera: Psychodidae) in an area of Atlantic Forest tree species in a metropolitan region in northeastern Brazil. J Vector Ecol 32: 336-41.
27. Oliveira CD, Assunção RM, Reis IA, Proietti FA (2001) Spatial distribution of human and canine visceral leishmaniasis in Belo Horizonte, Minas Gerais State, Brasil, 1994-1997. Cad Saude Publica 17: 1231-1239.
28. de Araújo VE, Morais MH, Reis IA, Rabello A, Carneiro M (2012) Early clinical manifestations associated with death from visceral leishmaniasis. PLoS Negl Trop Dis 6: e1511.
29. Pennisi MG, Hartmann K, Lloret A, Addie D, Belák S, et al. (2013) Leishmaniosis in cats: ABCD guidelines on prevention and management. J Feline Med Surg 15: 638-42.
30. Costa TAC, Rossi CN, Laurenti MD, Gomes AAD, Vides JP, et al. (2010) Ocorrência de leishmaniose em gatos de área endêmica para leishmaniose visceral. Braz J Vet Res Anim Sci 47: 213–217.
31. Coelho WM, do Amarante AF, Apolinário Jde C, Coelho NM, de Lima VM, et al. (2011) Seroepidemiology of Toxoplasma gondii, Neospora caninum, and Leishmania spp. infections and risk factors for cats from Brazil. Parasitol Res 109: 1009-1013.
32. Spada E, Proverbio D, Migliazzo A, Pepa AD, Perego R, et al. (2013) Serological and molecular evaluation of Leishmania Infantum in Stray Cats in a Nonendemic Area in Northern Italy. ISRN Parasitol: 6.
33. Dias Fde O, Lorosa ES, Rebêlo JM (2003) Blood feeding sources and peridomiciliation of Lutzomyia longipalpis (Lutz & Neiva, 1912) (Psychodidae, Phlebotominae). Cad Saude Publica 19: 1373-1380.
34. Savani ESMM, Camargo MCGO, Carvalho MR, Zampieri RA, Dos Santos MG, et al. (2004) The first record in the Americas of an autochthonous case of Leishmania (Leishmania) infantum chagasi in a domestic cat (Felix catus) from Cotia Country, São Paulo State, Brazil. Vet. Parasitol 120: 229–233.
35. Schallig HD, da Silva ES, Van Der Meide WF, Schoone GJ, Gontijo CM (2007) Didelphis marsupialis (common opossum): a potential reservoir host for zoonotic leishmaniasis in the metropolitan region of Belo Horizonte (Minas Gerais, Brazil). Vector Borne Zoo Dis 7: 387-93.
36. Humberg RM, Oshiro ET, Cruz Mdo S, Ribolla PE, Alonso DP, et al. (2012) Leishmania chagasi in opossums (Didelphis albiventris) in an urban area endemic for visceral leishmaniasis, Campo Grande, Mato Grosso do Sul, Brazil. Am J Trop Med Hyg 87: 470-472.
37. Cabrera MA, Paula AA, Camacho LA, Marzochi MC, Xavier SC, et al. (2003) Canine visceral leishmaniasis in Barra de Guaratiba, Rio de Janeiro, Brazil: assessment of risk factors. Rev Inst Med Trop Sao Paulo 45: 79-83.
38. Santiago ME, Vasconcelos RO, Fattori KR, Munari DP, Michelin Ade F, et al. (2007) An investigation of Leishmania spp. in Didelphis spp. from urban and peri-urban areas in Bauru (São Paulo, Brazil). Vet Parasitol 150: 283-290.
39. Ogrzewalska M, Uezu A, Labruna MB (2011) Ticks (Acari: Ixodidae) infesting wild birds in the Atlantic Forest in northeastern Brazil, with notes on rickettsial infection in ticks. Parasitol Res 108: 665-670.