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NORBERTO ASSIS MEMBRIVE

Considerações sobre *Leishmania* sp., flebotomíneos (Diptera: Phychodidae) e animais silvestres em área de preservação e leishmaniose cutânea disseminada no sul do Brasil

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde do Centro de Ciências da Saúde da Universidade Estadual de Maringá, como requisito parcial para a obtenção de qualificação para defesa do título de Doutor em Ciências da Saúde.

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Orientador: Prof. Dr. Ueslei Teodoro

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RESUMO

Na América Latina, a incidência de leishmanioses, especialmente a tegumentar (LT), é elevada, com destaque para o Brasil. A leishmaniose disseminada (LD) é uma forma grave com expressão incomum e ocorre em 2% dos casos de LT. Os objetivos deste estudo foram: 1) compreender melhor a dinâmica de *Leishmania*, flebotomíneos e reservatórios em área endêmica de LT, no sítio São Domingos, município de Arapongas, estado do Paraná, Brasil; 2) descrever um caso de LD que ocorreu no município de Sabaúdia, estado do Paraná. Os flebotomíneos foram coletados em tocas de animais silvestres, residência e interior da mata, com armadilhas luminosas de Falcão (FA), Shannon (SH) e armadilha piramidal quadrangular (QP). Foram realizadas pesquisas de *Leishmania* em flebotomíneos, amostras biológicas de roedores silvestres e cães por PCR e culturas "in vivo" e "in vitro"; pesquisa direta em lesões de pele animal, e IFI em amostras de sangue de cães. Foram coletados 88 flebotomíneos com armadilhas FA e 526 com armadilha SH, com predominância de *Pintomyia fischeri*. Com armadilha QP foram coletados 601 espécimes de *Brumptomyia brumpti* em buracos de tatu. Foram capturados 17 roedores silvestres, seis deles com lesões cutâneas com características de infecção por *Leishmania,* mas todos os testes foram negativos para *Leishmania*. A presença de espécies de animais silvestres e flebotomíneos no Sítio São Domingos, embora tenha mostrado resultados negativos nos testes realizados não se deve excluir a permanência do ciclo de *Leishmania* na área de preservação do sítio São Domingos. Desta forma, a vigilância epidemiológica deve ser mantida, uma vez que a distância entre as residências dos moradores e a mata é de 50 metros. O extraordinário número de 1.119 lesões de LD observado no paciente do município de Sabáudia constituiu-se no fato mais importante para o relato do caso. Além disto, este foi o primeiro relato desta forma de leishmaniose no sul do Brasil. O diagnóstico foi realizado pela detecção e isolamento de *Leishmania (Viannia) braziliensis* de lesões do paciente. O tratamento com antimoniato de meglumina foi bem sucedido e a resposta à medicação foi rápida, considerando que o paciente teve o diagnóstico da LD somente cinco meses após o início da primeira lesão.

Palavras-chave: Leishmaniose cutânea. Animais. Insetos Vetores. Epidemiologia.

Considerations on *Leishmania* sp., Sand flies (Diptera: Phychodidae) and wild animals in a preservation area and disseminated cutaneous leishmaniasis in southern Brazil

SUMMARY

In Latin America, the incidence of leishmaniasis, especially the tegument (TL), is high, especially in Brazil. Disseminated leishmaniasis (DL) is a severe form with uncommon expression and occurs in 2% of LT cases. The objectives of this study were: 1) to better understand the dynamics of *Leishmania*, sandflies and reservoirs in endemic area of TL, in the São Domingos ranch, Arapongas municipality, Paraná state, Brazil; 2) to describe a case of DL that occurred in the municipality of Sabaúdia, state of Paraná. The sand flies were collected in wild animal burrows, residence, and the forest, with Falcão light trap (FA), Shannon trap (SH) and quadrangular pyramidal trap (QP). Were performed *Leishmania* search in sand flies, biological samples of wild rodents and dogs by PCR and culture; parasite DS in animal skin lesions; infection of gold hamsters; and IIF in samples of dogs' blood. Were collected 88 sand flies with FA traps, and 526 sand flies with SH trap, with the predominance of *Pintomyia fischeri*. Were collected 601 specimens of *Brumptomyia brumpti* in armadillo burrows, with QP trap. Were captured 17 wild rodents, six of them had skin lesions with characteristics of *Leishmania* infection. Even was not find any positive test for *Leishmania*, epidemiological surveillance should be maintained, remembering that the human buildings are situated only 50 meters from the forest. Considering the species of wild animals and sandflies found in the São Domingos, the negative test found do not exclude the existence of the *Leishmania* cycle in this preservation area. The extraordinary number of 1,119 lesions observed in the patient is reported to be the most important fact for this case of the Sabaúdia municipality. In addition, this was the first report of this form of leishmaniasis in southern Brazil. The diagnosis was made by the detection and isolation of *Leishmania (V.) braziliensis* from lesions of the patient. Meglumine antimoniate treatment was successful and the response to medication was rapid, considering that the patient was diagnosed with the disease only five months after the onset of the first lesion.

***Keywords*:** Cutaneous leishmaniasis. Hosts. Vectors. Epidemiology.

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SUMÁRIO

1. CAPÍTULO I

1.1 INTRODUÇÃO

A leishmaniose tegumentar (LT) constitui um problema de saúde pública em 85 países, distribuídos em quatro continentes (Américas, Europa, África e Ásia), com registro anual de 0,7 a 1,3 milhão de casos novos (WHO, 2017). É considerada pela Organização Mundial da Saúde (OMS) como uma das seis mais importantes doenças infecciosas, pelo seu alto coeficiente de detecção e a capacidade de produzir deformidades (BRASIL, 2017). As formas clínico-epidemiológica da doença diferem de acordo com as espécies de *Leishmania*, a fauna de flebotomíneos e de mamíferos, a flora, as características ambientais, o clima, a topografia e as ações humanas sobre o meio ambiente (FORATINI, 1973, 1976; ARAGÃO & LIMA, 1987).

Nos ambientes naturais, o ciclo biológico de protozoários do gênero *Leishmania* envolve hospedeiros vertebrados (mamíferos) e invertebrados (flebotomíneos) silvestres (GRIMALDI et al., 1987, 1989). As espécies de *Leishmania* são parasitos extremamente bem sucedidos e as infecções naturais são encontradas em diferentes ordens de mamíferos (LAINSON & SHAW, 1987). A adaptação destes hospedeiros nos ambientes antrópicos, onde há também formação do ciclo biológico de *Leishmania* no peridomicílio, nas zonas rurais e urbanas, facilita a infecção do homem e de animais domésticos, sobretudo cães e equídeos (AGUIAR et al., 1986, 1989; ; PIRMEZ et al., 1988; LONARDONI et al., 1993). A *Leishmania (Viannia) braziliensis* foi a primeira espécie descrita e incriminada como agente etiológico da LT, caracterizada com a espécie mais importante, não só no Brasil, mas em toda a América Latina (BRASIL, 2017). Essa espécie tem sido mais isolada em cães domésticos e raramente encontrado em animais silvestres (GRIMALDI et al., 1989).

A LT ocorre em todos os estados do Brasil e acomete pessoas de todas as faixas etárias, de ambos os sexos. A doença humana é caracterizada por úlcera cutânea, única ou múltipla, cuja principal complicação é a metástase, por via hematogênica, para as mucosas da nasofaringe, com destruição desses tecidos, forma mucosa da doença (BRASIL, 2017). No estado do Paraná, foram relatadas infecções humanas por *L. (V.) braziliensis* e raras por *Leishmania (Leishmania) amazonensis*, por Silveira et al. (1990). Cepas de *L. (V.) braziliensis* foram isoladas de cães do estado do Paraná e identificadas por Lonardoni et al (1993) e Silveira et al. (1996).

A notificação da LT em diversos municípios do norte do Paraná, incluindo o

município de Arapongas, mostra a necessidade de estudos epidemiológicos sobre a diversidade da fauna e a frequência de flebotomíneos no domicílio, em abrigos de animais domésticos e nas matas remanescentes, especialmente nas localidades onde essa doença é endêmica (MEMBRIVE et al., 2012). Estabelecer medidas que tenham como objetivo a profilaxia ou prevenção de um determinado agravo à saúde requer conhecimentos prévios de sua história natural da doença (FORATTINI, 1976). As alterações antrópicas provocadas pelo homem no ambiente natural têm sido constantes e mostra a necessidade de investigações sobre a coexistência das populações humanas, de reservatórios e vetores, e suas implicações na epidemiologia da LT, sobretudo nas áreas endêmicas.

1.2 EPIDEMIOLOGIA

No Continente Americano há registro de casos de LT no extremo sul dos Estados Unidos e em todos os países da América Central e do Sul, exceto no Chile e do Uruguai (BRASIL, 2017). No Brasil, a LT é uma doença com diversidade de agentes, de reservatórios e de vetores que apresentam diferentes padrões de transmissão e um conhecimento ainda limitado sobre alguns aspectos, o que a torna de difícil controle (BRASIL, 2017). A LT é uma das afecções dermatológicas que merece muita atenção, pela amplitude de sua distribuição e pelo risco de causar deformidades no homem, principalmente nos casos de infecção por *L. (L.) amazonensis* (cutânea, disseminada e difusa) e *L.(V.) braziliensis* (cutânea disseminada e mucosa). A LT no Brasil afeta ambos os sexos e todas as faixas etárias, predominando em maiores de 10 anos, com 90% dos casos, e no sexo masculino, em 74% (BRASIL, 2017).

No Estado do Paraná, a LT foi descrita no início do século XIX e no século passado houve muitos relatos de casos até 1958 (LIMA et al, 1958; SILVEIRA et al, 1990). Houve um período de quiescência da doença até 1980, tornando-se endêmica desde então (VALE & FURTADO, 2005). Neste estado LT foi distribuída, segundo Monteiro et al. (2009) em dois circuitos de produção da doença: circuito Paraná-Paranapanema, onde se destacam os pólos Cinzas-Laranjinha, Tibagi, Ivaí - Pirapó, Piquiri e Baixo Iguaçu, e circuito Ribeira, onde se destaca o pólo Alto Ribeira. No Estado do Paraná são propostos dois circuitos de produção da doença: circuito Paraná, Ivaí - Pirapó – Tibagi, Circuito Paranapanema 3, Paranapanema 2, Paranapanema 1 e Cinzas, Circuito no Rio Paraná e Circuito Ribeira.

Nos municípios de importância epidemiológica na produção de LT no Paraná fica evidente que as ações antrópicas no ambiente, a urbanização crescente, os movimentos migratórios e as pressões socioeconômicas contribuíram para a expansão de áreas endêmicas,

surgindo focos da doença em zonas urbanas e localidades rurais onde a doença tem ocorrido historicamente, em áreas com preservação de pequenos trechos de cobertura florestal (NEGRÃO et al, 2013).

De acordo como o Ministério da Saúde (BRASIL, 2017) existem três perfis epidemiológicos característicos que prevalecem na transmissão das leishmanioses no Brasil: a) ambiente silvestre: neste padrão, a transmissão ocorre em área de vegetação primária e é fundamentalmente uma zoonose de animais silvestres, que pode acometer o ser humano quando este entra em contato com o ambiente silvestre, onde esteja ocorrendo enzootia; b) ocupacional e lazer: este padrão de transmissão está associado à exploração desordenada da floresta e derrubada de matas para construção de estradas, usinas hidrelétricas, instalação de povoados, extração de madeira, desenvolvimento de atividades agropecuárias, de treinamentos militares e ecoturismo; c) rural e urbano em áreas de colonização: este padrão está relacionado ao processo migratório, ocupação de encostas e aglomerados em centros urbanos associados a matas secundárias ou residuais.

1.3 ETIOLOGIA

Leishmania são parasitos digenéticos pertencem à ordem Trypanosomatida e família Trypanosomatidae, sendo agrupados e classificados em dois subgêneros de acordo com o desenvolvimento do parasito no invertebrado: *Leishmania* (ROSS, 1903) e *Viannia* (LAINSON & SHAW, 1987). As espécies do subgênero *Leishmania* desenvolvem-se na porção média e anterior do intestino do vetor (seção Suprapylaria), enquanto as do subgênero *Viannia* desenvolvem-se nas partes anterior, média e também no intestino posterior, na região do piloro (seção Peripylaria). O parasito apresenta dois estágios no seu ciclo de vida: a forma promastigota, com motilidade flagelar, que vive no trato digestivo de flebotomíneos, e a forma amastigota, não móvel, que habita macrófagos de hospedeiros vertebrados (GRIMALDI et al., 1991; CUPOLILLO et al., 2000).

No Velho Mundo há cinco espécies de agentes etiológicos de leishmanioses predominantes*: Leishmania Leishmania major, Leishmania Leishmania tropica, Leishmania Leishmania aethiopica, Leishmania Leishmania donovani e Leishmania Leishmania infantum,* as três primeiras causam leishmaniose cutânea e as duas últimas a leishmaniose visceral (ASHFORD, 1996; SHAW, 2007).

No Novo Mundo, são várias as espécies patogênicas de *Leishmania.* No Brasil sete espécies são causadoras da LT: *Leishmania (V.) braziliensis, Leishmania (Viannia)*

guyanensis, Leishmania (Viannia) lainsoni, Leishmania (Viannia) naiffi, Leishmania (Viannia) shawi, Leishmania (L.) amazonensis (LAINSON & SHAW, 1998*), Leishmania (Viannia) lindenbergi* (SILVEIRA et al., 2002) e *Leishmania (Leishmania) chagasi* causadora da leishmaniose visceral (SHAW, 2007).

Os relatos da autoctonia da LT no Novo Mundo baseiam-se na observação de faces deformadas de imagens humanas originárias (huacos) de populações moches dos Andes e Peru (400 a 900 a.C.), que se assemelham às lesões observadas em pacientes com leishmaniose cutâneo-mucosa (LAINSON & SHAW, 1998; ALTAMIRANO-ENCISO, 2003).

1.4 RESERVATÓRIO E VETORES

1.4.1 Hospedeiros vertebrados

Infecções por espécies de *Leishmania* que causam a LT foram descritas em várias espécies de animais silvestres, sinantrópicos e domésticos envolvendo canídeos, felídeos e equídeos (GRIMALDI & TESH, 1993). Desde as primeiras décadas do século passado, pesquisadores em todo o mundo buscam descobrir os reservatórios primários das leishmanioses, levando em conta o reservatório como um sistema ecológico no qual o agente infeccioso persiste indefinidamente (ASHFORD, 1996; ASHFORD, 2000). Os parasitos transmitidos por vetores ocorrem em uma ou mais espécies de vetores e mamíferos vivem em condições de densidade populacional e proximidade espacial, de modos que o agente possa ser indefinidamente transferido entre eles (ASHFORD, 1996).

O primeiro estudo demonstrando a importância dos roedores na epidemiologia da leishmaniose ocorreu no Panamá, onde Hertig, (1956) detectou o parasito *Leishmania (V.) braziliensis* em hemocultura, em 10% de 110 exemplares de ratos das espécies (*Proechimys semispinosus e Hoplomys gymminurus*) sem nenhuma lesão aparente. Forattini et al., (1960, 1972, 1973) conseguiram isolar *Leishmania* de infecções em roedores utilizando cultura de sangue e pele, comprovando a hipótese de que espécies de *Leishmania* do Novo Mundo possuem hospedeiros de habitats florestais onde mantém o ciclo enzoótico e expandem-se para o homem e animais domésticos, no ciclo antropozoonótico. O tipo de lesão pode ser interpretado como índice de adaptação do parasito ao hospedeiro, de modo que, quanto maior for a adaptação, menor será a gravidade da lesão, podendo atingir um estado de equilíbrio em que não haverá mais manifestação aparente e os animais silvestres infectados desempenhariam a função de reservatório do parasito (LAINSON et al., 1981). LAINSON &

SHAW (1992) reforçam que, entre animais silvestres, a infecção tende a ser benigna e inaparente, sugestiva de uma relação equilibrada resultante de uma antiga associação entre parasito e hospedeiro.

Algumas espécies de roedores, marsupiais, desdentados, canídeos silvestres e animais domésticos já foram registrados como hospedeiros e possíveis reservatórios naturais. Os animais domésticos (caninos, felinos e equinos) que se infectam por *L. (V.) braziliensis*, por não serem capazes de manter o ciclo epidemiológico em um ecótopo, são considerados hospedeiros secundários ou acidentais (LAINSON & SHAW, 1998; REITHINGER, 1999; MADEIRA et al., 2003; SCHUBACH et al., 2004; FALQUETO, 2005). A LT nestes animais pode apresentar-se como uma doença crônica com manifestações semelhantes às da doença humana, ou seja, o parasitismo ocorre preferencialmente na pele e em mucosas das vias aerodigestivas superiores (BRASIL, 2017). Há estudos que consideram a possibilidade de caninos e equídeos ser considerados reservatórios de *L. (V.) braziliensis* em ambiente peridoméstico (CRUZ et al., 1989; REITHINGER, 2002; REITHINGER et al., 2003). Em área endêmica de LT, onde há infecção canina, cresce cada vez mais o risco de infecção humana (MEMBRIVE et al., 2012).

Na Amazônia Brasileira, diversas pesquisas mostraram a infecção natural de animais por distintas espécies de *Leishmania*: *L. (V.) guyanensis* em *Tamandua tetradactyla* (tamanduá-mirim) (LAINSON et al., 1981), *L. (V.) naiffi* em *Dasypus novemcinctus* (tatu-galinha) (GRIMALDI, TESH, 1993), *L. (V.) shawi* em *Cebus apela* (macaco-prego), *Chiropotes satanus* (caxiú-preto), *Chloepus didactylus* (preguiça de dois dedos), *Bradypus tridactylus* (preguiça de três dedos) e *Nasua nasua* (quati) (GRIMALDI, 1993; LAINSON et al., 1989).

No Novo Mundo, mais de 40 espécies de mamíferos de várias ordens são consideras hospedeiros de *Leishmania* spp. em diferentes ciclos silvestres de transmissão. Contudo, poucas espécies são consideradas como reservatório principal no ciclo de transmissão natural da doença (GRIMALDI et al., 1993).

No Brasil, foram encontradas evidências de infecção natural de *Leishmania braziliensis* em várias espécies de roedores dos gêneros *Oryzomys* (LAINSON et al., 1981; LAINSON & SHAW, 1989), *Akodon* (BRANDÃO-FILHO et al., 2003; FORATTINI et al., 1972), *Holochilus* (BRANDÃO-FILHO et al., 2003), *Proechimys* (LAISON & SHAW, 1973), *Rattus* (BRANDÃO-FILHO et al., 1994; LAINSON & SHAW, 1979; VASCONCELLOS et al., 1994), *Rhipidomys* (LAINSON et al., 1981), *Nectomys* (BRANDÃO-FILHO et al., 1994),

Bolomys (BRANDÃO-FILHO et al., 1994) e o marsupial *Didelphis* (LAINSON & SHAW, 1973).

A maioria das áreas endêmicas de LT está intimamente associada com áreas florestais, via de regra com pouca densidade populacional. Guimarães et al. (1968) observaram que quando do desbravamento de zonas desérticas e o povoamento humano, surgem focos endêmicos rurais de LT, e mais tarde suburbanos e até mesmo, excepcionalmente, urbanos, com a participação de flebotomíneos peridomiciliários e cães como reservatórios secundário e talvez até o próprio homem.

Lainson & Show (1998) descreveram que as alterações dos meio ambientes decorrentes das ações antrópicas para a exploração de recursos naturais e agrícola, modificaram a epidemiologia da LT, possibilitando o surgimento de novas áreas endêmicas. A diversidade epidemiológica, a negligência em relação às leishmanioses no contexto da saúde pública e as dificuldades no controle da doença mostram a importância de estudos eco-epidemiológicos que envolvam hospedeiros, reservatórios e vetores de *Leishmania*.

1.4.2 Hospedeiros invertebrados

Os flebotomíneos são insetos dípteros que pertencem a família Psychodidae, subfamília Phlebotominae, e estão agrupados nos gêneros: *Phlebotomus, Sergentomyia e Chinius* no Velho Mundo, e *Lutzomyia, Brumptomyia* e *Warileya*, no Novo Mundo (YOUNG et al., 1994). E segundo Galati (2003) na subfamília Phlebotominae, os gêneros *Phlebotomus, Lutzomyia, Nyssomia, Pintomyia, Migonemyia,* são os que apresentam maior importância médica, pela ampla distribuição geográfica e pela capacidade de transmitir *Leishmania*. São conhecidas mais de 900 espécies destes insetos no mundo, 500 estão na América com aproximadamente metade no Brasil, mas apenas 30 delas têm importância vetorial (GALATI, 2003). Algumas espécies além de transmitirem *Leishmania,* são hospedeiras de cerca de 150 microrganismos, como tripanossomatídeos, vírus e bactérias (SHAW, 2003).Os flebotomíneos são insetos holometábolos, as larvas desenvolvem-se e alimentam-se de matéria orgânica depositada no solo, enquanto os adultos de ambos os sexos, se alimentam de açúcares de plantas. Somente as fêmeas adultas são hematófagas, sendo o alimento importante para maturação dos ovos. Os flebótomos possuem hábitos crepusculares e noturnos para realizar a hematofagia, embora possuam também hábitos matutinos e vespertinos no interior das matas (FORATTINI, 1973).

Em relação ao hábito alimentar as espécies ecléticas tem maior potencial de se

infectar e transmitir parasitos do grupo *Leishmania* devido ao fato de se alimentarem em fontes sanguíneas variadas, incluindo o homem, o cão e outros animais domésticos e silvestres, a exemplo de *Nyssomia longipalpis.* Outras espécies com características alimentares similares são *Nyssomyia intermedia, Migonemyia migonei* e *Pintomyia fischeri* que são muito frequentes em abrigos de animais e no domicílio humano (BRAZIL et al., 1991; RANGEL et al., 2009).

Várias espécies de flebotomíneos têm sido envolvidas na transmissão de *Leishmania* no Brasil, ressaltando-se como principais transmissores *Ny. intermedia, Mg. migonei, Nyssomia whitmani, Nyssomia umbratilis, Nyssomia wellcomei,* e *Nyssomia flaviscutellata* (RANGEL & LAINSON, 2003). O encontro de infecção natural de *Migonemyia neivai* (PITA-PEREIRA et al., 2009), *Ny. whitmani* (LUZ et al., 2000), *Pi. fischeri* (ROCHA et al., 2010), reforçaram a suspeita de participação destas espécies na veiculação de *Leishmania* (*V.*) *braziliensis* e, consequentemente, na transmissão da LTA.

1.5 JUSTIFICATIVA

A amplitude da distribuição das leishmanioses em 98 países evidencia a sua importância na saúde pública mundial (WHO, 2016). Na América Latina, a incidência de leishmanioses, especialmente a tegumentar (LT), é elevada, com destaque para o Brasil. Foram notificados 537.092 casos em todos os estados, no período de 1994 a 2014. No sul do país ocorreram 11.082 (2,15 %) casos, especialmente no estado do Paraná, que detém 93,0% deles e tem registro de LT em 316 municípios de 399 existentes (BRASIL, 2017).

No Brasil, a LT tem como agentes etiológicos diversas espécies de *Leishmania* e, entre estas, as mais importantes são *L. (L.) amazonensis, Leishmania (Viannia) guyanensis e L. (V.) braziliensis (*GRIMALDI et al, 1989*).* Os reservatórios das duas primeiras espécies já foram identificados, mas o mesmo não ocorre em relação à última espécie que talvez ocupe maior área geográfica (LAINSON, 1998).

No Estado do Paraná, a epidemiologia da LT vem sendo estudada desde 1986, contribuindo para melhorar o conhecimento da fauna de reservatórios, o comportamento de flebotomíneos, o controle destes insetos, o diagnóstico humano e canino, e o tratamento (SILVEIRA et al., 1999; LUZ et al., 2000; TEODORO et al., 2003; MEMBRIVE et al., 2004; 2006; LONARDONI et al., 2006; VELASQUEZ et al., 2006; SCODRO et al., 2008; HOFFMANN et Al., 2012; MEMBRIVE et al.,2012).

A relação vetor-hospedeiro é de grande interesse epidemiológico e biogeográfico, uma vez que o conhecimento do hábito alimentar, dos vetores e dos animais mamíferos procurados pelos vetores como fontes sanguíneas permite também o conhecimento da participação e contribuição para a manutenção de ambos os ciclos de *Leishmania*. Miranda et al. (1998) argumentam que a fonte de alimentos e outros parâmetros ecológicos determinam a distribuição e ocorrência da doença. Diversas espécies de animais silvestres como roedores marsupiais e edentados, e domésticos (cães, gatos e equídeos) já foram encontradas infectadas com *Leishmania* (FORATTINI, 1960; 1972; 1973; FALQUETO et al., 1986; BARRAL et al., 1986; CORREDOR et al., ROCHA et al., 1988; 1989; SILVEIRA et al., 1991; GRIMALDI et al., 1992; LLANOS-CUENTAS et al., 1999; LIMA et al., 2002; BRANDÃO et al., 2003; FIGUEIREDO et al., 2008; LAINSON, 2010;; DAHROUNG et al., 2010).

Os indicadores epidemiológicos obtidos com uma determinação mais detalhada de espécies de vetores e reservatórios primários em ambientes antrópicos poderão subsidiar informações para o desenvolvimento de métodos de controle.

1.6 OBJETIVOS

1.6.1 Geral

Avaliar a dinâmica de *Leishmania*, flebotomíneos e reservatórios em área endêmica de LT.

1.6.2 Específicos

Estudar e caracterizar a fauna de flebotomíneos, determinando as espécies vetoras de *Leishmania* em ecossistema endêmico;

Detectar a presença de *Leishmania* sp. em flebotomíneos utilizando a técnica de PCR;

Realizar o levantamento da fauna de mamíferos silvestres, visando identificar possíveis reservatórios naturais de *Leishmania* em ecossistemas degradados e preservados;

Verificar a dispersão de flebotomíneos em ecótopos naturais em relação à habitação humana, onde cães e humanos já foram diagnosticados com infecção por *Leishmania;*

Relatar os aspectos clínicos do primeiro caso de LD no Estado do Paraná.

1.7 REFERÊNCIAS

ALTAMIRANO-ENCISO, A.J. Sobre a origem e dispersão das leishmanioses cutânea e mucosa com base em fontes históricas pré e pós colombianas. História, Ciências e Saúde. **Editora Manguinhos**, v. 10, p. 853-882, 2003.

ASHFORD, R.W. The leishmaniases as emerging and reemerging zoonoses**. International, Journal for Parasitology**, v. 30, p. 1269-1281, 2000.

ASHFORD, R.W. Leishmaniasis reservoirs and their significante in control**. Clinics in Dermatology**, v. 14, p. 523-532, 1996.

AGUIAR, G.M.; SCHUBACK, P.A.; VILELA, M.L.; AZEVEDO, A.C.R. Aspectos ecológicos dos flebotomíneos do Parque Nacional da Serra dos Órgãos, Rio de Janeiro. II. Distribuição vertical (*Diptera, Psychodidae, Phlebotominae*). **Memórias do Instituto Oswaldo Cruz**, v.80, p. 187-194, 1986.

AGUIAR, G.M.; FERREIRA, V.A.; VILELA, M.L. Ecologia dos flebotomíneos em um recente foco ativo de leishmaniose tegumentar no norte do estado do Paraná (*Diptera, Psychodidae, Phebotominae*), **Memórias do Instituto Oswaldo Cruz**, v. 80 p. 7-8, 1989.

ARAGÃO, M.M.; LIMA, L.C. Sobre a dispersão de Lutzomya intermedia (Diptera:Psychodidae). **Caderno de Saúde Pública,** v.4, p. 473-479, 1987.

BARRAL, A,; BADARÓ, R.; BARRAL-NETTO, M,; GRIMALDI, JR. G.; MOMEN, H.; CARVALHO, EM. Isolation of *Leishmania mexicana amazonensis* from the bone marrow in a case of American visceral leishmaniasis. **American Journal of Tropical Medicine and Hygiene.** v. 35, p.732-7344, 1986.

BRANDÃO, F.S.P.; BRITO M.E,; CARVALHO, F.G.; ISHIKAWA, E.A.; CUPOLILLO, E.; FLOETER-WINTER, L. Wild and synanthropic hosts of *Leishmania* (*Viannia*) *braziliensis* in the endemic cutaneous leishmaniasis locality of Amaraji, Pernambuco State, BRAZIL. **Transactions of the Royal Society of Tropical Medicine ad Hygiene**, v. 97, p. 291-296, 2003.

BRANDÃO-FILHO S.P. et al. American cutaneous leishmaniasis in Pernambuco, Brazil: Eco-epidemiological aspects in 'Zona da Mata' region, **Memórias do Instituto Oswaldo Cruz,** Rio de Janeiro, v. 89, p. 445-449, 1994.

BRAZIL, R P.; MORTON, I.E.; WARD, R.D. Notes of the feeding habits of Lutzomyia (N) withmani (Diptera: Psychodidae). **Memórias do Instituto Oswaldo Cruz**, v. 86, p. 497-498, 1991.

BRASIL. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual de Vigilância da Leishmaniose Tegumentar Americana. 2. ed atual. Brasília, DF: **Editora do Ministério da Saúde**, 2017.

BRASIL. Ministério da Saúde. Sistema Nacional de Agravo de notificações. http://www2.datasus.gov.br/DATASUS/index.php , acesso em 20-12-2016.

CORREDOR, A.; GALLEGO, J.F.; TESH, R.B.; MORALES, A.; CARRASQUILLA, C.F.; YOUNG, D.G. *Didelphis marsupialis*, an apparent wild reservoir of *Leishmania donovani chagasi* in Colombia, South America. **Journal of Tropical Medicine and Hygiene**, v. 40, p. 480-486, 1989. **American**

CRUZ, M.A. Zoonotic cutaneous leishmaniasis due to Leishmania (Viannia) braziliensis associated with domestic animals in Venezuela and Brazil**. Memórias do Instituto Oswaldo Cruz**, v. 84, p. 19-28, 1989.

CUPOLILLO, E. A revised classification for Leishmania and Endotrypanum. **Parasitology Today**, v.16, p. 142-144, 2000.

DAHROUG, M.A.; ALMEIDA, A.B.; SOUSA, V.R.; DUTRA, V.; TURBINO, N,C.; NAKAZATO, L. *Leishmania* (*Leishmania*) *chagasi* in captive wild felids in Brazil. **Transactions of the Royal Society of Tropical Medicine ad Hygiene, v.** 104, p. 73-74., 2010.

FALQUETO, A. Participação do cão no ciclo de transmissão da leishmaniose tegumentar no município de Viana, estado do Espírito Santo, Brasil. **Memórias do Instituto Oswaldo,** v. 81, p. 155-163, 1986.

FALQUETO, A.; FERREIRA, A.L. Reservatórios e Transmissão de Leishmanias. In: COURA, J R. **Dinâmica das Doenças Infecciosas e Parasitárias**. v. 5, p. 739-752, 2005.

FORATTINI, O.P.; PATTOLI, D.B.G.; RABELO, E.X.; FERREIRA O.A. Nota sobre infecção natural de *Oryzomys capito laticeps* em foco enzoótico de leishmaniose tegumentar no estado de São Paulo, Brasil. **Revista de Saúde Pública,** v.7, p.181-184, 1973.

FORATTINI, O.P. Sobre os reservatórios naturais da leishmaniose tegumentar americana. **Revista do Instituto de Medicina Tropical**, v. 2, p. 195-203, 1960.

FORATTINI, O.P.; RABELLO, E.X.; SERRA, O.P.; COTRIN, M.D.; GALATI, E.A.B.; BARATA, J.M.S. Observações sobre a transmissão da leishmaniose tegumentar no Estado de São Paulo, Brasil. **Revista de Saúde Pública**, v.10, p. 31-43, 1976.

FORATTINI, O.P.; PATTOLI, D.B.; RABELLO, E.X.; FERREIRA, O.A. Infecções naturais de mamíferos silvestres em área endémica de leishmaniose tegumentar do Estado de São Paulo, Brasil. **Revista de Saúde Pública,** v. 6, p. 255-261, 1972.

FIGUEIREDO, F.B,; GREMIÃO, I.D,; PEREIRA, S.A.; FEDULO, L.P.; MENEZES, R.C.; BALTHAZAR, D.A. First report of natural infection of a bush dog (*Speothos venaticus*) with *Leishmania* (*Leishmania*) *chagasi* in Brazil. **Transactions of the Royal Society of Tropical Medicine ad Hygiene**, v. 102, p. 200-201, 2008.

GALATI E.A.B.; Morfologia e Taxonomia. In: E.F. Rangel, and R. Lainson . Flebotomíneos do Brasil. Rio de Janeiro: **Editora Fiocruz**; 2003.

GIL, L.H.; BASANO, S.A,; SOUZA, A.A,; SILVA, M.G,; BARATA, I.; ISHIKAWA, E.A. Recent observations on the sand fly (Diptera: Psychodidae) fauna of the State of Rondônia, western Amazónia, Brazil: the importance of *Psychodopygus davisi* as a vector of zoonotic cutaneous leishmaniasis. **Memória do Instituto Oswaldo Cruz**, v. 98, p.751-755, 2003.

GUIMARÃES, F.N.; AZEVEDO, M.; DAMASCENO, R. Leishmaniose tegumentar - zoonose de roedores silvestres na Amazônia. **Memórias do Instituto Oswaldo Cruz**, v. 66, p. 151-168, 1968.

GRIMALDI, J.; DAVID, J.R.; MCMAHON, D.P. Indentification and distribution of new world *Leishmania* species characterized by serodene analysis using monoclonal anti-bodies. **American Journal of Tropical Medicine and Hygiene**, v. 36, p. 270-287, 1987.

GRIMALDI, J.; DAVID, J.R.; MCMAHON, D.P. A review of the geographic distribution and epidemiology of leishmaniasis in the new world. **American Journal of Tropical Medicine and Hygiene,** v. 41, p. 687-725, 1989.

GRIMALDI, G.; KREUTZER, R.D.; HASHIGUCHI, Y.; MIMORY, T.; TESH, R.B. Description of *Leishmania equatorensis* sp.n (Kinetoplastida: Trypanosomatidae), a new parasite

infecting arboreal mammals in Ecuador. **Memória do Instituto Oswaldo Cruz,** v87, p. 221-228, 1992.

GRIMALDI, G. et al. Characterization and classification of leishmanial parasites from humans, wild animals, and sand flies in the Amazon region of Brazil. **American Journal of the Tropical Medicine and Hygiene,** v. 44, p. 645-661, 1991.

GRIMALDI-JR, G.; TESH, R.B. Leishmaniasis of the New World: Current concepts and implications for future research. **Clinical Microbiology Reviews**, v. 6, p. 230-250, 1993.

HOFFMANN, A. R.; NAVARRO, I. T.; CAMARGO JUNIOR, V. E.; CALDART, E. T.; MITSUKABREGANÓ, R.; PEREIRA, P. M. Leishmania amazonensis em cão com quadro clínico de leishmaniose visceral no Estado do Paraná, Brasil – relato de caso. **Semina: Ciências Agrárias**, Londrina, v. 33, n. 6, p. 3265-3270, 2012.

LONARDONI, M.V.C.; TEODORO, U.; ARRAES, S.M.A.A.; SILVEIRA, T.G.V. Leishmaniose canina no noroeste do Paraná, sul do Brasil. **Revista de Saúde Pública**, v. 27, p. 378-379, 1993.

LONARDONI, M.V.C.; SILVEIRA, T.G.V.; MEMBRIVE, N.A.; RODRIGUES, R.; ZANZARINI, P.; ISHIKAWA, E.; TEODORO, U. Human and canine american cutaneous leishmaniasis in Mariluz, Paraná state, Brazil. **Caderno de Saúde Pública,** v. 22, p. 2713-2716 , 2006.

LAINSON, R.; SHAW, J.J,; SOUZA, A.A. A,; SILVEIRA, F.T,; FALQUETO, A. Further observations on *Lutzomyia ubiquitalis* (Psychodidae: Phlebotominae), the sand fly vector of *Leishmania (Viannia) lainsoni*. **Mem Inst Oswaldo Cruz,** v. 87, p. 437-439, 1992.

LAINSON, R.; SHAW, J.J. New World leishmaniasis. The neotropical *Leishmania* species. In: COLLIER, L., BALOWS, A., SUSSMAN, M. (Eds.). **Topley & Wilson' s Microbiologand Microbial Infectious Diseases**, v. 5, p. 241-266, 1998.

LAINSON, R.; SHAW, J.J. *Leishmania (Viannia) naiffi sp*. n., a parasite of the armadillo, *Dasypus novemcinctus* (L.) in Amazonan Brazil. **Annales de Parasitologie Humaine et Comparee,** v. 64, p. 3-9, 1989.

LAINSON, R. The Neotropical Leishmania species: a brief historical review of their discovery, ecology and taxonomy. **Revista Pan Americana de Saude**, v. 1, p. 13-23, 2010.

LAINSON, R.; SHAW, J.J. The role of animals in the epidemiology of South American leishmaniasis. In WHR Lumsden, DA Evans, *Biology of the Kinetoplastida,* **Academic Press,** v. 2, p. 1-116, 1979.

LAINSON, R. Epidemiologia e ecologia de leishmaniose tegumentar na Amazônia. **Hiléia Médica**, v.3, p. 35-40, 1981.

LAINSON, R.; BRAGA, R.R.; SOUZA, A.A.; POVOA, M.M.; ISHIKAWA, E.A.; SILVEIRA, F.T. *Leishmania* (*Viannia*) *shawi* sp.n., a parasite of monkeys, sloths and procyonids in Amazonian Brazil. **Annales de Parasitologie Humaine et Comparee,** v. 64, p. 200-207, 1989.

LAINSON, R.; SHAW, J.J.; PÓVOA, M. The importance of edentates (sloths and anteaters) as primary reservoirs of *Leishmania braziliensis guyanensis*, causative agent of "pian-bois" in North Brazil. **Transactions of the Royal Society of Tropical Medicine ad Hygiene,** v. 75, p. 611-612, 1981.

LAINSON, R.; SHAW, J. J.; SILVEIRA, F. T.; BRAGA, R. R. American visceral leishmaniasis: on the origin of *Leishmania* (*Leishmania*) *chagasi*. **Transactions of the Royal Society of Tropical Medicine ad Hygiene**, v. 3, p. 81-517, 1987.

LAINSON, R.; SHAW, J.J.; WARD, R.D.; FRAIHA, H. Leishmaniasis in Brazil. IX. Considerations on the *Leishmania braziliensis* complex: importance of sandflies of the' genus *Psychodopygus* (Mangabeira) in the transmission of *L. braziliensis* in north Brazil. **Transactions of the Royal Society of Tropical Medicine ad Hygiene,** v. 67, p. 184-196, 1973.

LIMA, H.; GUGIELMO, Z.; RODRÍGUEZ, A.; CONVIT, J.; RODRIGUEZ, N. Cotton rats (*Sigmodon hispidus*) and black rats (*Rattus rattus*) as possible reservoirs of *Leishmania* spp. in Lara State, Venezuela. **Memória do Instituto Oswaldo Cruz**, v. 97, p. 169-174, 2002.

LIMA, E.C.; LUZ, E.; SOUZA, L.A. Leishmaniose tegumentar americana no município de Foz do Iguaçu. **Revista Médica Paraná**, v. 27, p. 53-58, 1958.

LLANOS-CUENTAS, E.A.; RONCAL, N.; VILLASECA, P.; PAZ, L.; OGUSUKU, E.; PEREZ, J.E. Natural infections of *Leishmania peruviana* in animals in the Peruvian Andes. **Transactions of the Royal Society of Tropical Medicine ad Hygiene,** v. 93, p.15-20, 1999.

LUZ, E.; MEMBRIVE, N A.; CASTRO E.A.; TOMAZ-SOCCOL, V. *Lutzomyia whitmani* (Diptera: Psychodidae) as vector of *Leishmania braziliensis* in Paraná state, southern Brazil. **Annals of Tropical Medicine & Parasitology**, v. 94, p. 623-631, 2000.

MADEIRA, M.F. *Leishmania* (*Viannia*) *braziliensis* in naturally infected dogs. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 36, p. 551-555, 2003.

MEMBRIVE, N.A.; RODRIGUES, G.; LONARDONI, M.V.C.; SILVEIRA, T.G.V.; TEODORO, U. Flebotomíneos de municípios do Norte do Estado do Paraná, sul do Brasil. **Entomologia Vectores,** v. 11, p. 673-680, 2004.

MEMBRIVE, N.A.; RODRIGUES, G.; LONARDONI, M.V.C.; TEODORO, U.; SILVEIRA, T.G.V. Environmental and animal characteristics as factors associated with American cutaneous leishmaniasis in rural locations with presence of dogs, Brazil. **Plos One,** 2012.

MONTEIRO, W.M. Mobilidade populacional e produção da leishmaniose tegumentar americana no Estado do Paraná, sul do Brasil. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 42, p. 509-514, 2009.

MIRANDA, C.; MASSA, J.L.; MARQUES, C.C.A. Sensoriamento remoto orbital como recurso para análise da ocorrência da leishmaniose tegumentar americana em localidade urbana da região Sudeste do Brasil. **Revista de Saúde Pública**, v. 32, p.455-63, 1998.

NEGRÃO, G.N.; FERREIRA, M.E.M.C. Circuitos espaciais da leishmaniose tegumentar americana no estado do Paraná. **Revista Brasileira de Geografia Médica e da Saúde**, v. 9, p. 74-94, 2013.

PIRMEZ, C., COUTINHO, S.G.; MARZORCHI, M.C.A.; GRIMALDI, J. Canine american cutaneous leishmaniasis: A clinical and immunological study in dogs naturally infected with *Leishmania Braziliensis Braziliensis* in an endemic area of Rio de Janeiro, Brazil. **American Journal of Tropical Medicine and Hygiene**, v. 38, p. 52-5, 1988.

PITA-PEREIRA, D.; SOUZA, G. D.; ZWETSCH, A.; ALVES, C.R.; RANGEL, E.F. First report of *Lutzomyia* (*Nyssomyia*) *neivai* (Diptera: Psychodidae: Phlebotominae) naturally infected by *Leishmania (Viannia) braziliensis* in a periurban area of South Brazil using a multiplex polymerase chain reaction assay. **American Journal of Tropical Medicine and Hygiene**, v. 80, p. 593-595, 2009.

RANGEL, E.F.; LAINSON, R. Proven and putative vectors of American cutaneous leishmaniasis in Brazil: aspects of their biology and vectorial competence. **Memória do Instituto Oswaldo Cruz.** v**.** 104, p. 937-954, 2009.

RANGEL, E F.; LAINSON, R. Ecologia das Leishmanioses: Transmissores de Leishmaniose Tegumentar Americana. In: RANGEL, E. F.; LAINSON, R. Flebotomíneos do Brasil. 1. **Editora Fiocruz**, v.68, p. 291-309, 2003.

ROCHA, N. M.; MELO, M. N.; BABÁ, E. H.; DIAS, M.; MICHALICK, M. S.; COSTA, C. A. *Leishmania braziliensis braziliensis* isolated from *Akodon arviculoides* captured in Caratinga, Minas Gerais, Brazil. **Transactions of the Royal Society of Tropical Medicine ad Hygiene,** v. 82, p. 1-68, 1988.

ROCHA, L.S. Survey of natural infection by Leishmania in sand fly species collected in southeastern Brazil. **Transactions of the Royal Society of Tropical Medicine and Hygiene,** v. 104, p. 461-466, 2010.

ROSS, R. Note on the bodies recently described by Leishman and Donovan**. British Medical Journal**, v. 2, p. 1261-1262, 1903.

REITHINGER, R.. Evaluation of PCR as a diagnostic mass-screening tool to detect Leishmania (Viannia) spp. in domestic dogs (Canis familiaris). **Journal of Clinical Microbiology**, v. 41, p. 1486-1493, 2003.

REITHINGER, R.; DAVIES, C.R. American cutaneous leishmaniasis in domestic dogs: an example of the use of the polymerase chain reaction for mass screening in epidemiological studies**. Transactions of the Royal Society of Tropical Medicine ad Hygiene,** v.96, p. 123-126, 2002.

REITHINGER, R.; DAVIES, C.R. Is the domestic dog (*Canis familiaris*) a reservoir host of American cutaneous leishmaniasis? A critical review of the current evidence. **American Journal of Tropical Medicine and Hygiene,** v. 61, p. 530-541, 1999

SCODRO, R.B.J.; CASTRO, K.R.R.; NEITZKE, H.C.; MEMBRIVE, N.A.; KUHL, J.B.; SILVEIRA, T.G.V.; TEODORO, U. Investigation of Natural Infection by Leishmania in Sandflies, **Brasilian Archives of Biology and Technology**, v. 51, p. 483-491, 2008.

SILVEIRA TOBIAS, T.; LAINSON, R.; SHAW, J.J.; BRAGA, R. R.; ISHIKAWA, E.A.Y.; SOUZA, A.A.A. Leishmaniose cutânea na Amazônia: isolamento de *Leishmania* (*Viannia*) *lainsoni* do roedor *Agouti paca* (Rodentia: Dasyproctidae) no Estado do Pará, Brasil. **Revista do Instituto de Medicina Tropical de São Paulo,** v. 33, p. 18-22, 1991.

SILVEIRA, F.T. An outbreak of cutaneous leishmaniasis among soldiers in Belém, Pará State, Brazil, caused by *Leishmania* (*Viannia) lindenbergi* n. sp. A new leishmanial parasite of man in the Amazon Region. **Parasite**, v. 9, p. 3-50, 2002.

SILVEIRA, T.G.V.; LORNADONI, M.V.C.; BERNA, F.H.Z.; ANTUNES, V.; TEODORO, U.; JORGE, F.A.; ZANZARINI. P.D. Investigação sorológica em cães de área endêmica de leishmaniose tegumentar, no Estado do Paraná, Sul do Brasil. **Caderno de Saúde Pública**, v. 12, p. 89-93, 1996.

SILVEIRA, T.G.V.; TEODORO, U.; ARRAES, S.M.; LONARDONI, M.L.; SHAW, J.J.; ISHIKAWA, E.A.; LAIRSON, R. An autochthonous case of cutaneous leishmaniasis caused by *Leishmania (Leishmania) amazonensis* Lainson & Shaw, 1972, from the north of Paraná State, Brazil. **Memórias do Instituto Oswaldo Cruz**, v. 85, p. 475-476, 1990

SILVEIRA, T.G.V.**;** ARRAES, S.M.A.A.; BERTOLINI, D.A.; TEODORO, U**.** Observações sobre o diagnóstico laboratorial e a epidemiologia da leishmaniose tegumentar no estado do Paraná, sul do Brasil. **Revista da Sociedade Brasileira de Medicina Tropical,** v. 32, p. 413-423, 1999.

SHAW, J.J. The leishmaniases - survival and expansion in a changing world. A mini review. **Memórias do Instituto Oswaldo Cruz**, v. 102, p. 541-547, 2007.

SHAW, J.J. Transmissão de colaboradores agentes: Os flebotomíneos brasileiros como hospedeiros e vetores de determinadas espécies. In: RANGEL, E. F.; LAINSON, R. Flebotomíneos do Brasil. Rio de Janeiro, **Editora: Guanabara Koogan**, v. 12, p. 337-351, 2003.

SCHUBACH, T.M.P. American cutaneous leishmaniasis in two cats from Rio de Janeiro, Brazil: first report of natural infection with *Leishmania* (*Viannia*) *braziliensis*. **Transactions of the Royal Society and Tropical Medicine Hygiene**, v. 98, p. 165-167, 2004.

TEODORO, U.; SILVEIRA, T.G.V.; SANTOS, D.R.; OLIVEIRA, O. Influência da reorganização, da limpeza e da desinsetização de edificações na densidade populacional de flebotomíneos, no município de Doutor Camargo, estado do Paraná, Brasil. **Caderno de Saúde Pública**, v. 19, p. 1801-1813, 2003.

TEODORO, U.; SANTOS, D.R.; OLIVEIRA, S.; NEITZKE, H.C.; MONTEIRO, W.M.; LONARDONI, M.V.C.; SILVEIRA, T.G.V. Informações preliminares sobre flebotomínios do norte do Paraná. **Caderno de Saúde Pública**, v. 40, p. 1-4, 2006.

VALE, E.C.S.; FURTADO, T. Tegumentary leishmaniasis in Brazil: a historical review related to the origin, expansion and etiology. **Anais Brasileiros de Dermatologia,** v. 80, p. 421-428, 2005.

VASCONCELOS, I.A.B. The identity of *Leishmania* isolated from sand flies and vertebrate hosts in a major focus of cutaneous leishmaniasis in Baturite, Northeast Brazil. **American Journal of Tropical Medicine and Hygiene,** v. 50, p. 158-164, 1994.

VELASQUEZ, L.G.; MEMBRIVE, N.A.; LONARDONI, M.V.; RODRIGUES, G.; TEODORO, U.; SILVEIRA, T.G.V. PCR in the investigation of canine american tegumentary leishmaniasis in northwestern Paraná state, Brazil. **Caderno de Saúde Pública**, v. 22, p. 571-578, 2006.

World Health Organization, leishmaniansis. Available http://www.who.int/leishmaniasis/en/. (Accessed 2017 Fev 20).

YOUNG, D.G. & DUNCAN, M.A. Guide to the identification andgeographic distribution of *Lutzomyia* sandflies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). **Memoirs of the American Entomological Institute**, v. 54, p. 1- 881, 1994.

CAPITULO II

Artigo 1: Considerations of potential vectors and animal reservoirs in an emerging cutaneous leishmaniasis area in São Domingos ranch, Paraná State in Southern Brazil

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Considerations of potential vectors and animal reservoirs in an emerging cutaneous leishmaniasis area in *São Domingos* ranch, *Paraná State* in Southearn Brazil

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ABSTRACT

The aim of this study was to better understand the dynamics of *Leishmania* sand flies and reservoirs in *São Domingos* ranch, Arapongas municipality, *Paraná State*, an anthropic environment in an endemic area of cutaneous leishmaniasis (CL). Sand flies were collected in wild animal burrows, residences and in the forest, with *Falcão* light trap (FA), Shannon trap (SH) and quadrangular pyramidal trap (QP). The search for *Leishmania* was made on sand flies, biological samples of wild rodents and dogs using PCR and culture; while parasite direct search (DS) was carried out on animal skin lesions; infection of gold hamsters; and indirect immunofluorescence (IIF) test in dog blood samples. Eighty eight (88) sand flies were collected with FA traps and 526 sand flies using the SH trap, with a predominance of *Pintomyia fischeri*. Six hundred and one (601) specimens of *Brumptomyia brumpti* were collected in armadillo burrows, with the QP trap. Seventeen (17) wild rodents were captured, six of them had skin lesions with characteristics of Leishmania infection. Even though no positive test was found for *Leishmania*, epidemiological surveillance should be maintained, remembering that the human buildings are situated only 50 m from the forest. Considering the species of wild animals and sandflies found in *São Domingos*, the negative test found do not exclude the existence of the *Leishmania* transmission cycle in this preservation area.

KEYWORDS: Cutaneous leishmaniasis. Hosts. Vectors. Epidemiology.

INTRODUCTION

The distribution of leishmaniasis in 98 countries shows its importance in world public health[1]. In Latin America, the incidence of leishmaniasis, especially cutaneous leishmaniasis (CL), has been increasing and in Brazil, 635,399 cases all over the States were reported from 1990 to 2013[2]. During this period, in the Southern region of the country, there were 13,889 (2.2%) cases, with 94.9% in *Paraná State*, in 316,399 municipalities[2].

In South, Central and North America, the evolutionary cycle of several *Leishmania* species in natural environments comprises wild terrestrial and arboreal mammals (Rodentia, Edentata, Marsupialia and Primates) and sand flies from several species[3-6]. The cohabitation of different species of sand flies, reservoirs and parasites in most distinct environments comprises a complex ecological scenario that makes it difficult to understand the epidemiology of leishmaniasis. Out of approximately 900 phlebotomine sand flies species identified in the world, 500 are in America with roughly half in Brazil[6], but only 30 of them exhibit vectorial importance[7-10].

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The vertebrate vector/host relationship is a determinant factor in the *Leishmania* cycle due to the need of the vector to seek in mammal animals, the food (blood) that allows it to reproduce, suggesting that the sources of food and ecological factors determine the geographical distribution of vectors, hosts, and parasites[11].

In areas of old colonization associated with remaining modified forests, there is evidence of sand fly adaptation and *Leishmania* wild reservoirs, providing the protozoa cycle formation of this genus in the peridomiciliary and rural areas and urban center outskirts[12-14]. This fact explains the endemic CL persistence in these areas. The detection of several sand fly species[15-17], wild animals[17-20] and domestic animals (dogs, cats, and horses)[13,14,21,22] infected by *Leishmania* in anthropic environments, explains partially, CL persistence in these environments.

The coexistence of human populations, reservoirs and *Leishmania* vectors in anthropic areas requires investigations to verify the implications of changes in CL epidemiology in endemic areas with the same characteristics. As the anthropic process in the natural environment is constant in *Paraná State* investigations in endemic areas of old colonization can contribute to the design of the most appropriate procedures for the control of this parasitosis.

Membrive *et al*.[14] verified 41 human CL cases in rural locations of the Arapongas municipality, from 1999 to 2008. In eight of the studied locations, only human cases occurred while in six of the locations, both human and canine cases occurred[14]. The *São Domingos* ranch was one of the studied rural locations; it is an area that suffered successive alterations, and where four human CL cases occurred in 2007 and five dogs had canine CL in 2008. Even though no human case occurred in the last few years, those facts led to the development of this study in *São Domingos* ranch, aiming a better understanding the dynamics of *Leishmania*, sand flies and reservoirs in an anthropic environment in an endemic area of CL.

MATERIALS AND METHODS

Characteristics of the Arapongas municipality and description of the study area

The study was conducted in the *São Domingos* ranch, located at 23° 29'50.17"S and 51° 27'47.50" W, Arapongas municipality, Northern central *Paraná State*, Southern Brazil. It has a subtropical climate, with a maximum temperature of 32 °C and a minimum of 8 °C and lowered plateau of plain and little-dissected tops[14]. *São Domingos* ranch is a permanent preservation area with approximately 2.1 ha, rocky soil, sharp slope and visibly degraded primary forest. The human buildings are situated 50 m away from the forest. The residents cultivate soybeans, corn, wheat, tomatoes and work with poultry farming.

Sand fly collection and identification

For the collection of sand flies, *Falcão* light traps (FA)[23], Shannon light traps (SH)[24] and a quadrangular pyramidal trap (QP) were used (Figure 1).

Figure 1 - Quadrangular pyramidal trap (QP), especially developed to collect these insects in wild animal burrows

FA traps: These traps operate overnight without human presence, and its installation is easy in diversified environments (domiciles, domestic animal shelters, forests and others). Sand flies were collected with one FA trap installed on the porch of a residence and another in the dog shelter, from 6:00 pm to 8:00 am, four times a week, in December 2014 (224 hours) and February 2015 (224 h). Two FA traps were installed inside the forest, from 6:00 pm to 8:00 am once a week, from March to May and from August to November 2015, totaling 392 h of collection per trap.

SH trap: Sand flies were captured inside the forest with this trap, next to a bamboo bush, from 6:00 pm to 12:00 pm, once a month, in March, April, May, August, September and October 2015, totaling 36 h of captures. The SH trap attracts sand fly species with some degree of anthropophilia due to human presence during insects collection.

QP trap: The homemade QP trap, especially designed to collect insects in wild animal burrows, was constructed as follows: (i) a quadrangular structure in the base (40×40 cm) and a rectangular form in the top (20×30 cm), joined by iron bars with 70 cm of length; (ii) this structure was covered with white cloth and has two opening sides that allow the hand passage for handling the Castro-type suction tube[25] for the sand fly collection that would emerge from

inside the burrow into the trap; (iii) the bottom of the trap is hollow and the top is closed with transparent glass for insect viewing . This trap was placed over the entrance of the wild animal burrows. To promote the insect collection with the QP trap, a small tree branch was introduced inside the burrow, and circular movements were carried out. Due to the lack of clarity inside the forest, the collections were made during the day and a flashlight was needed to visualize the sand flies inside the QP. In each burrow, at the end of the collection, the suction tube was labeled with the ecotope number, sealed with nylon mesh for ventilation, packed in a thermal box with ice and transported to the laboratory, where the sand flies were dissected and identified.

The area where the sand flies were collected with the QP was delimited and divided into 40 sectors, each with 500 m^2, to facilitate the finding of natural ecotopes (wild animal burrows, especially armadillos), which were numbered in wooden stakes and georeferenced for their monitoring. Two hundred and six (206) wild animal burrows were found. In each of them, a collection period was carried out from 8:00 am to 12:00 am, three days a week, during the months of September and October, 2014. Given that the sand flies were captured in only six burrows (4, 24, 108, 109, 145, and 158) after this period, the collections were maintained only in these six burrows, one day in the first week of each month, from 8:00 am to 12:00 am, from November 2014 to October 2015.

Photographic record

The six burrows where the sand flies were collected were monitored with two digital cameras (Bushnell® USA) for ten consecutive days, in February and March 2016, to verify the presence of armadillos and other mammals. Each burrow was monitored for 240 h.

The collected sand flies were kept alive until their processing in the laboratory. The male sand flies were preserved in 70% ethanol and subsequently stained and identified. The females were kept alive for *Leishmania* search by dissection and identification of the species according to the genitalia morphology[26]. The flagellates detected in the sand fly digestive tract were cultivated in 199 medium (Invitrogen), containing 10% inactivated fetal bovine serum and antibiotics for *Leishmania* isolation. The cultures were incubated at 27 °C and observed for four weeks to check eventual parasite growth.

The female sand flies in which no flagellates were found, but containing blood in their digestive tract, were conserved in tubes with isopropanol, each with 1 to 14 specimens of the same species and from the same ecotope, for subsequent *Leishmania* DNA detection by PCR.

The insects were processed at the Laboratory of Medical Entomology of the *Secretaria Municipal de Saúde of Arapongas*. Then, it was followed by nomenclature[6] and abbreviations[27] of sand flies. The nomenclature of the species follows Galati[6], and the abbreviations of the genera follow Marcondes[27].

Capture and collection of biological material from wild mammals

Wild rodents were captured in bamboo bushes, with four traps of the type "live rat traps" (Havahart, USA) in the month of November 2015. A total of 720 h of capture per trap was carried out, with an inspection performed every 48 h.

The captured rodents were anesthetized with ketamine and examined for the presence of suspected CL lesions[26]. The suspected rodents were euthanized for the collection of biological materials (blood, skin, spleen and liver) for culture, and preparation of slides for microscopy, PCR and hamster infection. The rodents without suspected lesions were returned to their original environment after the capture period.

For *Leishmania* research by PCR, specific primers targeting the parasite kDNA were used. Liver, spleen and skin fragments intended for PCR were kept in STE buffer (NaCl 0.1 M; Tris 10 mM; pH 8.0; $Na_2EDTA.2H_2O$ 1 mM; pH 8.0), and preserved at 30 ºC until DNA extraction.

Collection of dog biological material

A sample of blood (5 mL) was collected from 10 brachial vein of the dog to perform the indirect immunofluorescence test (IIF). Serum samples were stored at -20 °C. The IIF for leishmaniasis was performed using *L. (V.) braziliensis* promastigotes- and anti-dog immunoglobulin G conjugated to fluorescein (Sigma), considering titles > 1:40 significant.

The biopsy was performed to collect tissue fragments of the dogs with cutaneous lesions suggestive of CL, after lesion asepsis and topical 1% xylocaine inoculation, for parasite direct search (DS) and parasite isolation in gold hamster (*Mesocricetus auratus*). For the DS, fragments were smeared on slides, stained using the Giemsa method and examined under an optical microscope.

Leishmania detection in sand flies by multiplex PCR- and in biological samples from wild mammals by conventional PCR

DNA extraction of sand flies was done according to Oliveira *et al*.[28]. The DNA extraction of wild animals'

biopsy samples was performed by kit Puregene® (Gentra - USA), according to the manufacturer's instructions. For multiplex PCR, two pairs of primers were used: A1 and A2, which amplify a fragment of 110-120 bp of the conserved region of DNA from the minicircle of the kinetoplast (kDNA) of the genus *Leishmania*[29] and 5Llcac and 3Llcac, which amplify a fragment of 220 bp from the *IVS6* gene region of the cacophony in insects of the genus *Lutzomyia*[30]. The PCR reaction mixture (final volume 25 μL) was composed of 0.5 mM of each of the primers (Invitrogen), 0.24mM dNTP (Invitrogen), 1U Taq DNA Polymerase (Invitrogen), 1.5 mM $MgCl_2$, 1 × enzyme buffer, and 2 μL DNA template. The amplification was carried out in a G96G & G96GEN cycler (Biosystems) at 95 °C for 5 min for initial denaturation, followed by 35 cycles, each divided into three stages, of denaturation (30 sec at 95 °C), annealing (30 sec at 55 °C), and polymerization (30 sec at 72 °C). After this, the extension was continued for a further 10 min at 72 °C, and the tubes were then kept at 4 °C until analysis. This PCR reaction mixture and amplification were performed according to Santos BA *et al*. (unpublished data).

Two conventional PCR of biological material were performed from wild animals samples. For the first PCR, primers A1 and A2[29] were used. The second round of amplification was performed with the primers MP3H and MP1L, which amplify a fragment of 70 bp from the conserved region of the kinetoplast minicircle (kDNA) of the subgenus Leishmania (Viannia)[31].

In both PCR assays, DNA of *L. (V.) braziliensis* was used as the positive control while sterile water was used as the negative control.

Ethical aspects

The captures and procedures with wild animals were performed with the license approved by the *Environmental Institute of Paraná* (IAP) (protocol Nº 06/14). All procedures with hamsters were performed according to the protocols approved by the Ethics Committee on Animal Use from the *State University of Maringá* (CEUA-EMU) (protocol Nº 083/14).

Statistical analyses

The proportion and G tests were analyzed by the BioEstat software version 5.3, and the Mid-P exact test was performed by using the OpenEpi software version 3.1, while the level of statistical significance was set at $p < 0.05$.

RESULTS

Five sand flies were collected with FA traps in the porch of the residence (Table 1), and none in the dog shelter. In the forest, 83 sand flies were collected from the species *Pintomyia pessoai* (Barreto & Coutinho), *Pintomyia fischeri* (Pinto), *Nyssomyia whitmani* (Antunes & Coutinho), *Migonemyia migonei* (France), *Expapillata firmatoi* (Barretto, Martins & Pellegrino), and *Brumptomyia brumpti* (Larrousse); 21 (25.3%) of them were male and 63 (74.7%), female; from the six collected species, 52 (62.6%) were *Pi. fischeri* (Table 1). There was no difference in the number of sand flies collected per hour in relation to sex and environment (p=0.529). All females collected with FA trap were submitted to dissection for the search of flagellate forms. Flagellate forms were found only in one specimen of *Mi. migonei* collected in the forest. An aliquot of its digestive tract (0.1 mL) was subjected to multiplex PCR, with a negative result, and another aliquot (0.5 mL) was inoculated into hamster's hand paws but did not result in *Leishmania* isolation.

Table 1 - Sand flies collected with FA trap in *São Domingos* ranch, Arapongas municipality, *Paraná State*, Brazil

Species/Ecotope/Sex	Porch of the residence[1]			Forest[2]		
	Male	Female	Total	Male	Female	Total
Brumptomyia brumpti	0	0	0	0	4	4
Migonemyia migonei	0	1	1	0	3	3
Pintomyia fischeri	0	4	4	11	41	52
Nyssomyia whitmani	0	0	0	6	6	12
Expapillata firmatoi	0	0	0	0	2	2
Pintomyia pessoai	0	0	0	4	6	10
Total	0	5	5	21	62	83
%		100.0	100.0	25.3	74.7	100.0

1. Operating time of the FA trap in the porch of the residence: 448 hours. 2. Two FA traps were installed inside the forest. Operating time of each FA trap in the forest: 392 hours.

By using the SH trap, 526 sand flies of eight species were collected: *Pi. pessoai*, *Nyssomyia neivai* (Pinto), *Pi. fischeri*, *Ny. whitmani*, *Mi. migonei*, *Psatiromyia Shannoni* (Dyar), *Pintomyia monticola* (Costa Lima), and *Br. Brumpti*; 105 (20.0%) of them were male and 421 (80.0%) female (Table 2). The proportion of female was significantly higher than that of male (p <0.001). Of the total collected, 384 (73.0%) were *Pi. fischeri*; and from these, 81 (21.1%) were male and 303 (78.9%) female (Table 2). There was no difference in the species distribution and sex of sand flies (p=0.536).

Table 2 - Sand flies collected with SH trap in *São Domingos* ranch, Arapongas municipality, *Paraná State*, Brazil

Species/Sex	Male	Female	Total
Brumptomyia brumpti	0	2	2
Pintomyia monticula	0	2	2
Migonemyia migonei	3	23	26
Pintomyia fischeri	81	303	384
Nyssomyia whitmani	20	76	96
Migonemyia neivai	0	5	5
Psathyromyia shannoni	1	7	8
Pintomyia pessoai	0	3	3
Total	105	421	526

Operating time of the SH trap: 36 hours.

During the captures with SH trap, it was observed that *Pi. fischeri* specimens did not land on the trap walls, but on the bamboos, close to where the trap was installed, at the height of three to four meters, from 7:00 pm to 9:00 pm. After this hour, when the wind started blowing, these sand flies landed about one meter from the ground, always on the bamboos, where they were collected.

Six hundred and one (601) specimens of *Br. brumpti* were collected with the QP trap in six of the 206 wild animal burrows, of which 77.2% were male and 22.8% female (Table 3). All females (137) were dissected for the search of flagellate forms in the digestive tract and the salivary gland; none of them showed flagellate forms. The multiplex PCR for detection of *Leishmania* DNA was performed with 52 females of *Br. Brumpti* (nine pools), which contained blood in the digestive tract. All pools contained the 220 bp fragment from the *IVS6* gene region of the cacophony of the sand flies, but none contained the 110-120 bp fragment from the conserved region of the kinetoplast minicircle (kDNA) of the genus *Leishmania*. The digital cameras (Bushnell) revealed that the burrows were inhabited by armadillos *Dasypus novemcinctus*.

Table 3 - Sand flies of the species *Br. brumpti* collected with QP trap in six of 206 wild animal burrows found in the *São Domingos* ranch, Arapongas municipality, *Paraná State*, Brazil, from September 2014 to October 2015

Burrow number	Male	Female	Total
Burrow 4	42	7	49
Burrow 24	74	24	98
Burrow 108	132	47	179
Burrow 109	89	14	103
Burrow 119	73	28	101
Burrow 124	54	17	71
Total	464	137	601
%	77.2	22.8	100.0

Using the "live rat traps", 17 rodents were captured, from the species *Akodon* spp., *Oligoryzomys* spp., *Nectomys* spp., and *Oryzomys* spp. (Table 4). Two rodents *Akodon spp*. and two *Oligoryzomys spp*. had ear lesions; one specimen of

Table 4 - Results of test for *Leishmania* search in wild rodents and domestic dogs, carried out in the *São Domingos* ranch, Arapongas municipality, *Paraná State,* Brazil

Animals	CA (n)	AL (n)	Organ culture*			PCR*	IH*	DS*	IIF*
			Spleen	Liver	Skin				
Rodents									
Akodon sp.	6	2	0/2	0/2	0/2	0/4	0/2	0/2	0/0
Oligoryzomys spp.	4	2	0/2	0/2	0/2	0/4	0/2	0/2	0/0
Nectomys spp.	4	1	0/1	0/1	0/1	0/2	0/1	0/1	0/0
Oryzomys spp.	3	1	0/1	0/1	0/1	0/2	0/1	0/1	0/0
Dogs									
Canis familiaris	10	1	0/0	0/0	0/1	0/0	0/1	1/1	0/10
Total	27	7	0/6	0/6	0/7	0/6	0/7	1/7	0/10

CA=Captured animals; AL=Animals with lesion; IIF=Indirect immunofluorescence test; PCR=Polymerase chain reaction performed with a fragment of spleen and liver of each rodent with lesion; DS=parasite direct search performed in lesions; IH=Infection in hamster; *Positive or negative result/number of samples.

Oryzomys spp. had an unpigmented area in the tail, and the other had a nodule in the tail. One specimen of *Nectomys spp*. had a snout lesion and an unpigmented area in the tail. The culture of spleen, liver, and fragment of skin lesion; PCR for detection of *Leishmania* DNA in liver and spleen of rodents; infection in hamster, and DS of lesions were negative (Table 4).

The IIF results of the ten dogs was negative (Table 4). One of the ten dogs had ear lesions suggestive of CL, of which samples were collected to perform DS, culture in 199 medium and infection in hamster. Amastigotes were found in DS, but culture and infection in hamsters were negative (Table 4).

DISCUSSION

In the *São Domingos* ranch, the collected species of sand flies were *Br. brumpti*, *Ex. firmatoi*, *Mi. migonei*, *Ny. neivai*, *Ny. whitmani*, *Pi. fischeri*, *Pi. monticola*, *Pi. pessoai*, and *Ps. shannoni*, which have already been reported in the *Paraná State*[16,32-34]. The species *Mi. migonei*, *Ny. whitmani*, *Ny. neivai* and *Mi. migonei* have already been found to be naturally infected by *Leishmania* spp. in *Paraná State*[28,35] and other Brazilian States[15,16,36-44]. Despite the low levels of sand flies collected when compared to studies carried out in Northern *Paraná State*[16,26-29], the importance of this study lies in the observations that could be made, as in the same locality wild animals where captured, possibly representing *Leishmania* reservoirs and sand flies as well as dogs were examined.

The species of *Pi. fischeri* predominated in the collection with FA traps in the forest, constituting 62.6% from the total, and 86.5% of this species were female. Close to the same bamboos where these two traps were placed, 73.0% of the sand flies collected with SH were female *Pi. fischeri*. These data alongside with evidence that the natural breeding sites of *Pi. fischeri* occur on the ground, at the base of trees[45,46] allow us to consider the possibility that this species is associated with bamboo plantations, where they find shelter and conditions for procreation, with high humidity and organic matter in decomposition. Lainson *et al*.[47] suggested that *Pi. fischeri* is an example of sand fly that can adapt to environments modified by human action and is still able to transmit *L.(V.) braziliensis* to wild animals in secondary forests with fragments still preserved. Furthermore, it is possible that this sand fly species and the wild rodents occupy the same habitat given that the traps for the rodents were installed in the same bamboos.

However, only four sand flies were collected in the porch of the residence; this fact should not be disregarded given that *Pi. fischeri* has already been found to be infected by *Leishmania*[44]. Furthermore, it is highly anthropophilic, adapts well to domestic animal shelters and invade the domiciles to suck human blood[26,48]. For this reason, the possibility of the sand fly population increment, and the proximity of the forest with possible reservoirs of *Leishmania*, the actions to control sand fly populations should always be carried out, under the risk of CL cases increment.

In South America, especially in Brazil, sand flies of the genus *Brumptomyia*, rather than the *Dasypodidae*[49], have been commonly collected in animal burrows, indicating that there is a close association of this sand fly genus with armadillos. The armadillos (*Dasypus novemcinctus*) have already been identified as *Leishmania naiffi* hosts[25,50]. All *Br. brumpti* collected in armadillo burrows, in *São Domingos* ranch, were negative by dissection for the search of flagellate forms and also by PCR for *Leishmania* search.

Researchers have described wild rodents as reservoirs of *Leishmania*[17]. Barros *et al*.[51] captured 257 wild mammals from three orders (Rodentia, Marsupialia and Lagomorfa), five families and 15 species, but isolated *Leishmania* from only two rodents; and Gomes *et al*.[52] captured 36 rodents in an endemic area for CL, all with negative results. The small number of rodents captured in the forest show that these animals populations are low in the surveyed locality. This factor also limits the understanding of the role of wild rodents in the *Leishmania* transmission cycle. Besides that, the isolation rate of *Leishmania* in wild animals is very low and requires the capture of a large number of animals[51,52]. Despite the negative results of the PCR for *Leishmania* search in six of the 17 wild rodents captured, the skin lesions had characteristics of *Leishmania* infection.

In November 2014, part of the studied property was deforested and occupied by greenhouses devoted to fruit and vegetables cultivation, close to the remaining forest, where synthetic chemicals were used in pests management. This procedure may have affected the sand fly population, explaining the low number of insects collected.

Even if the IIF performed with dog blood samples were negative, and no positive test was found for *Leishmania*, epidemiological surveillance should be maintained with periodic examinations of domestic animals, remembering that the human buildings are situated only 50 m from the forest. Considering the species of wild animals and sandflies found in *São Domingos*, the negative test found do not exclude the existence of the *Leishmania* transmission cycle in this preservation area.

REFERENCES

1. World Health Organization. Leishmaniasis: situation and trends. [cited 2016 Mar 04]. Available from: http://www.who.int/gho/neglected_diseases/leishmaniasis/en/

2. Brasil. Ministério da Saúde. Casos de leishmaniose tegumentar americana: Brasil, grandes regiões e unidades federadas, 1990 a 2013. [cited 2016 Jan 18]. Available from: http://portalarquivos.saude.gov.br/images/pdf/2014/setembro/09/LT-Casos.pdf
3. Grimaldi G Jr, David JR, McMahon-Pratt D. Identification and distribution of New World Leishmania species characterized by serodeme analysis using monoclonal anti-bodies. Am J Trop Med Hyg. 1987;36:270-87.
4. Grimaldi G Jr, Tesh RB, McMahon-Pratt D. A review of the geographic distribution and epidemiology of leishmaniasis in the new world. Am J Trop Med Hyg. 1989;41:687-725.
5. Lainson R, Shaw JJ. Epidemiology and ecology of leishmaniasis in Latin-America. Nature. 1978;273:595-600.
6. Galati EA, Dedet JP, Vignes R, Rangel EF, Ready PD, Aguiar GM, et al. Morfologia e taxonomia. In: Rangel EF, Lainson R, organizadores. Flebotomíneos do Brasil. Rio de Janeiro: Fiocruz; 2003. p.23-206.
7. Aguilar CM, Fernández E, Fernández R, Cannova DC, Ferrer E, Cabrera Z, et al. Urban visceral leishmaniasis in Venezuela. Mem Int Oswaldo Cruz. 1998;93:15-6.
8. Young DG, Lawyer PG. New World vectors of the leishmaniases. In: Harris KF, editor. Current topics in vector research. New York: Springer; 1987. p.29-37.
9. Young DG, Duncan MA. Guide to the identification and geographic distribution of Lutzomyia sandflies in Mexico, the West Indies, Central and South America (Diptera:Psychodidae). Gainesville: American Entomological Institute; 1994.
10. Lainson R, Shaw JJ. New World Leishmaniasis. The neotropical Leishmania species. In: Collier L, Balows A, Sussman M, editors. Topley & Wilson's microbiology and microbial infections. 9th ed. London: Arnold; 1998. p.241-66.
11. Miranda C, Marques CC, Massa JL. Sensoriamento remoto orbital como recurso para análise da ocorrência da leishmaniose tegumentar americana em localidade urbana da região Sudeste do Brasil. Rev Saúde Pública. 1998;32:455-63.
12. Miranda C, Massa JL, Marques CC. Análise da ocorrência de leishmaniose tegumentar americana através de imagem obtida por sensoriamento remoto orbital em localidade urbana da região Sudeste do Brasil. Rev Saúde Pública. 1996;30:433-7.
13. Lonardoni MV, Teodoro U, Arraes SM, Silveira TG, Bertolini DA, Ishikawa EA, et al. Leishmaniose canina no noroeste do Paraná, sul do Brasil. Rev Saúde Pública. 1993;27:378-9.
14. Membrive NA, Rodrigues G, Gualda KP, Bernal MV, Oliveira DM, Lonardoni MV, et al. Environmental and animal characteristics as factors associated with American cutaneous leishmaniasis in rural locations with presence of dogs, Brazil. Plos One. 2012;7:e47050.
15. Pita-Pereira D, Souza GD, Zwetsch A, Alves CR, Britto C, Rangel EF. First report of Lutzomyia (Nyssomyia) neivai (Diptera: Psychodidae: Phlebotominae) naturally infected by Leishmania (Viannia) braziliensis in a periurban area of South Brazil using a multiplex polymerase chain reaction assay. Am J Trop Med Hyg. 2009;80:593-5.
16. Luz E, Membrive N, Castro EA, Dereure J, Pratlong F, Dedet JA, et al. Lutzomyia whitmani (Diptera: Psychodidae) as vector of Leishmania braziliensis in Paraná state, southern Brazil. Ann Trop Med Parasitol. 2000;94:623-31.
17. Lainson R. The Neotropical Leishmania species: a brief historical review of their discovery, ecology and taxonomy. Rev Pan-Amaz Saude. 2010;1:13-32.
18. Forattini OP. Sobre os reservatórios naturais da leishmaniose tegumentar americana. Rev Inst Med Trop São Paulo. 1960;2:195-203.
19. Sherlock IA, Miranda JC, Sadigursky M, Grimaldi Jr G. Natural infection of the opossum Didelphis albiventris (Marsupialia, Didelphidae) with Leishmania donovani, in Brazil. Mem Inst Oswaldo Cruz. 1984;79:511.
20. Rocha NM, Melo MN, Babá EH, Dias M, Michalick MS, Costa CA, et al. Leishmania braziliensis braziliensis isolated from Akodon arviculoides captured in Caratinga, Minas Gerais, Brazil. Trans R Soc Trop Med Hyg. 1988;82:68.
21. Silveira TG, Teodoro U, Lornadoni MV, Toledo MJ, Bertolini DA, Arraes SM, et al. Investigação sorológica em cães de área endêmica de leishmaniose tegumentar, no Estado do Paraná, Sul do Brasil. Cad Saúde Pública. 1996;12:89-93.
22. Zanzarini PD, Santos DR, Santos AR, Oliveira O, Poiani LP, Lonardoni MC, et al. Leishmaniose tegumentar americana canina em municípios do norte do Estado do Paraná, Brasil. Cad Saúde Pública. 2005;21:1957-61.
23. Falcão AR. Um novo modelo de armadilha luminosa de sucção para pequenos insetos. Mem Inst Oswaldo Cruz. 1981;76:303-5.
24. Shannon RC. Methods for collecting and feeding mosquitoes in jungle yellow fever studies. Am J Trop Med. 1939;19:131-40.
25. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Manual de vigilância da Leishmaniose Tegumentar Americana. 2ª ed. Brasília: Ministério da Saúde; 2007. [cited 2011 Mar 08]. Available from: http://bvsms.saude.gov.br/bvs/publicacoes/manual_vigilancia_leishmaniose_2ed.pdf
26. Forattini OP. Entomologia médica. São Paulo: Edgar Blücher: EDUSP; 1962-1973.
27. Marcondes CB. A proposal of generic and subgeneric abbreviations for phlebotomine sandflies (Diptera: Psychodidae: Phlebotominae) of the world. Entomol News. 2007;118:351-6.
28. Oliveira DM, Reinhold-Castro KR, Bernal MV, Legriffon CM, Lonardoni MV, Teodoro U, et al. Natural infection of Nyssomyia neivai by Leishmania (Viannia) spp. in the state of Paraná, southern Brazil, detected by multiplex polymerase chain reaction. Vector Borne Zoonotic Dis. 2011;11:137-43.
29. Romero GA, Guerra MV, Paes MG, Cupolillo E, Toaldo CB, Macêdo VO, et al. Sensitivity of the polymerase chain reaction

30. Lins RM, Oliveira SG, Souza NA, Queiroz RG, Justiniano SC, Ward RD, et al. Molecular evolution of the cacophony IVS6 region in sandflies. Insect Mol Biol. 2002;11:117-22.
31. Lopez M, Inga R, Cangalaya M, Echevarria J, Llanos-Cuentas A, Orrego C, et al. Diagnosis of Leishmania using the polymerase chain reaction: a simplified procedure for field work. Am J Trop Med Hyg. 1993;49:348-56.
32. Teodoro U, Santos DR, Silva AM, Massafera R, Imazu LE, Monteiro WM, et al. Fauna de flebotomíneos em municípios do norte pioneiro do estado do Paraná, Brasil. Rev Patol Trop. 2010;39:322-30.
33. Teodoro U, Silveira TG, Santos DR, Santos ES, Santos AR, Oliveira O, et al. Influência da reorganização, da limpeza do peridomicílio e da desinsetização de edificações na densidade populacional de flebotomíneos no Município de Doutor Camargo, Estado do Paraná, Brasil. Cad Saúde Pública. 2003;19:1801-13.
34. Membrive NA, Rodrigues G, Membrive U, Monteiro WM, Neitzke HC, Lonardoni MV, et al. Flebotomíneos de municípios do norte do estado do Paraná, sul do Brasil. Entomol Vect. 2004;11:673-80.
35. Neitzke-Abreu HC, Reinhold-Castro KR, Venazzi MS, Scodro RB, Dias AC, Silveira TG, et al. Detection of Leishmania (Viannia) in Nyssomyia neivai and Nyssomyia whitmani by multiplex polymerase chain reaction, in southern Brazil. Rev Inst Med Trop São Paulo. 2014;56:391-5.
36. Pita-Pereira D, Alves CR, Souza MB, Brazil RP, Bertho AL, Barbosa AF, et al. Identification of naturally infected Lutzomyia intermedia and Lutzomyia migonei with Leishmania (Viannia) braziliensis in Rio de Janeiro (Brazil) revealed by a PCR multiplex non-isotopic hybridisation assay. Trans R Soc Trop Med Hyg. 2005;99:905-13.
37. Córdoba-Lanús E, De Grosso ML, Piñero JE, Valladares B, Salomón OD. Natural infection of Lutzomyia neivai with Leishmania spp. in northwestern Argentina. Acta Trop. 2006;98:1-5.
38. Carvalho GM, Andrade Filho JD, Falcão AL, Lima AC, Gontijo CM. Naturally infected Lutzomyia sand flies in a Leishmania-endemic area of Brazil. Vector Borne Zoonotic Dis. 2008;8:407-14.
39. Marcondes CB, Bittencourt IA, Stoco PH, Eger I, Grisard EC, Steindel M. Natural infection of Nyssomyia neivai (Pinto, 1926) (Diptera: Psychodidae, Phlebotominae) by Leishmania (Viannia) spp. in Brazil. Trans R Soc Trop Med Hyg. 2009;103:1093-7.
40. Saraiva L, Carvalho GM, Gontijo CM, Quaresma PF, Lima AC, Falcão AL, et al. Natural infection of Lutzomyia neivai and Lutzomyia sallesi (Diptera: Psychodidae) by Leishmania infantum chagasi in Brazil. J Med Entomol. 2009;46:1159-63.
41. Carvalho MR, Valença HF, Silva FJ, Pita-Pereira D, Pereira TA, Britto C, et al. Natural Leishmania infantum infection in Migonemyia migonei (França, 1920) (Diptera: Psychodidae: Phlebotominae) the putative vector of visceral leishmaniasis in Pernambuco State, Brazil. Acta Trop. 2010;116:108-10.
42. Margonari C, Soares RP, Andrade-Filho JD, Xavier DC, Saraiva L, Fonseca AL, et al. Phlebotomine sand flies (Diptera: Psychodidae) and Leishmania infection in Gafanhoto Park, Divinópolis, Brazil. J Med Entomol. 2010;47:1212-9.
43. Rocha LS, Santos CB, Falqueto A, Grimaldi G Jr, Cupolillo E. Molecular biological identification of monoxenous trypanosomatids and Leishmania from antropophilic sand flies (Diptera: Psychodidae) in Southeast Brazil. Parasitol Res. 2010;107:465-8.
44. Pita-Pereira D, Souza GD, Pereira TA, Zwetsch A, Britto C, Rangel EF. Lutzomyia (Pintomyia) fischeri (Diptera: Psychodidae: Phlebotominae), a probable vector of American cutaneous leishmaniasis: detection of natural infection by Leishmania (Viannia) DNA in a specimens from the municipality of Porto Alegre (RS), Brazil, using multiplex PCR assay. Acta Trop. 2011;120:273-5.
45. Barretto MP, Coutinho JO. Contribuição ao conhecimento dos flebótomos de São Paulo. IV. Descrição de três novas espécies. Pap Avulsos Depart Zool. 1941;1:143-58.
46. Aguiar GM, Medeiros WM. Distribuição regional e habitats das espécies de flebotomíneos do Brasil. In: Rangel EF, Lainson R, organizadores. Flebotomíneos do Brasil. Rio de Janeiro: Fiocruz; 2003. p.207-55.
47. Lainson R. The American Leishmaniasis: some observation on their ecology and epidemiology. Trans Roy Soc Trop Med Hyg. 1983;77:569-96.
48. Brazil PR, Brazil BG. Biologia de flebotomíneos neotropicais. In: Rangel EF, Lainson R, organizadores. Flebotomíneos do Brasil. Rio de Janeiro: Fiocruz; 2003. p.257-309.
49. Mangabeira Filho O. 13ª Contribuição ao estudo dos Flebotomus (Diptera: Psychodidae): Flebotomus (Brumptomyia) travassosi Mangabeira 1942. Mem Inst Oswaldo Cruz. 1942;37:375-81.
50. Naiff RD, Freitas RA, Naiff MF, Arias JR, Barrett TV, Momen H, et al. Epidemiological and nosological aspects of Leishmania naiffi Lainson & Shaw, 1989. Mem Inst Oswaldo Cruz. 1991;86:317-21.
51. Barros GC, Sessa PA, Mattos EA, Carias VR, Mayrink W, Alencar JT, et al. Foco de leishmaniose tegumentar americana nos municípios de Viana e Cariacica, Estado do Espírito Santo, Brasil. Rev Saúde Pública. 1985;19:146-53.
52. Gomes AC, Coutinho SG, Paim GV, Oliveira SM, Galati EA, Nunes MP, et al. Aspectos ecológicos da leishmaniose tegumentar americana. Rev Inst Med Trop São Paulo. 1990;32:105-15.

Artigo 2: Disseminated cutaneous leishmaniasis caused by *Leishmania braziliensis* in Southern Brazil

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Disseminated cutaneous leishmaniasis caused by *Leishmania braziliensis* in Southern Brazil

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ABSTRACT

The authors report a case of disseminated cutaneous leishmaniasis caused by *Leishmania (Viannia) braziliensis*, in a 55 years old patient with 1,119 lesions distributed throughout the body. The patient resides in *Sabáudia* municipality, North of *Paraná* State, Southern Brazil, where there was no previous report of this form of leishmaniasis. Treatment with meglumine antimoniate was successful, although the diagnosis was made only five months later.

KEYWORDS: *Leishmania (Viannia) braziliensis.* Disseminated cutaneous leishmaniasis. *Paraná State, Brazil.*

INTRODUCTION

Leishmaniases are infectious diseases caused by protozoa of the genus *Leishmania*, with a wide spectrum of clinical manifestations, depending on the species of *Leishmania* involved[1]. Originally, leishmaniases were considered wild zoonoses, but have been reported in rural and urban areas where domestic animals, especially dogs, have been often diagnosed with *Leishmania* infection[2].

Disseminated cutaneous leishmaniasis (DL) caused by *Leishmania (Viannia) braziliensis* and *Leishmania (Leishmania) amazonensis* is a rare form of clinical manifestation and accounts for approximately 2% of the reported cases of cutaneous leishmaniasis (CL) in Brazil[2]. Torres[3] reported the first case of DL caused by *L. (V.) braziliensis* in the State of Bahia, Brazil. In 1986, Costa *et al.*[4] published the first case of CL. Since then, just a few cases[5] of DL, with additional research on the parasites characteristics, their immunological behavior and response of patients to treatment[2,5] have been reported. DL is characterized by expressing multiple papular acneiform lesions, compromising exposed areas, such as limbs, and frequently face and trunk[2,4,5]. The number of lesions can reach hundreds, according to the Ministry of Health[2].

Another atypical form of CL, known as difuse cutaneous leishmaniasis (DCL) or anergic-DCL, caused by *L. (L.) amazonensis*[2], was first reported by Silva[6], in Brazil, and Convit[7], in Venezuela. DCL is characterized by the presence of multiple non-ulcerated nodular lesions, weak response of T-cells to antigens of *Leishmania* parasites (amastigotes) and a large number of *Leishmania* inside macrophages[8]. DCL is also rare, with 1 or 2 cases diagnosed per year and the number of lesions can, as well, reach the hundreds[2,8]. These two clinical forms, DL and DCL, were precisely compared and differentiated based on reported cases by Hashiguchi *et al.*[9].

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This report deals with a case of DL in an inhabitant of Vila dos Crentes, a rural town in the municipality of *Sabáudia*, North of *Paraná State*, Southern Brazil, showing an extraordinary number of cutaneous lesions.

CASE REPORT

At the beginning of March 2015, the patient, a 55 years old driver, observed a lesion on the anterior surface of his leg, with a granular bottom and raised edges. After approximately three months of development of the primary lesion, small lesions appeared on the face and thereafter throughout the body (scalp, all the face, neck, arms, hands, including the palms, legs and the dorsal part of the foot), except in the palms surface and genital organ, totaling 1,119 lesions, counted with the a manual counter (Hand Held). The disease was identified five months after the onset. During this period, the patient had lost about 15 kg. When the first lesion appeared, the patient initially received an unsuccessful treatment for allergy. Thereafter, he was treated with six ampoules of penicillin G benzathine 1,200,000 IU, and had unsuccessful results once again. It is noteworthy that the municipality of *Sabaúdia* is located in the important center of *Ivai-Pirapó* from the circuit *Paraná-Paranapanema* of CL incidence, in the North-Central mesoregion of *Paraná State*[10].

Evolution of the case and laboratory examination

The patient fail to respond to treatment within three months of the disease onset, then he sought medical resources in the municipality of Arapongas, *Paraná*, where he was subjected to the following tests: V.D.R.L., negative; FTA-Abs IgG and IgM, negative; Herpes simplex Virus anti-IgG, positive (23.8 U/mL); Herpes simplex Virus anti-IgM, negative; Rubella anti-IgG, positive (73.7 IU/mL); Rubella anti-IgM, negative; Aspartate Aminotransferase (AST), normal; Alanine Aminotransferase (ALT), normal; Lactic dehydrogenase (LDH), normal; Gamma Glutamyl Transferase, normal; Alpha 1-Acid Glycoprotein, increased 164 mg/dL; Hepatitis B, negative; Hepatitis C, negative; Brucella anti-IgG and IgM, negative; Chlamydia trachomatis anti-IgG, reagent (1/80); Chlamydia trachomatis anti-IgM, negative; Complete blood count, normal; Anti-HIV, negative. The biopsy, performed in a private laboratory, showed the absence of viral infection, a favorable morphologic pattern in the diagnosis of a staphylococci and screening for fungi negative. The indirect immunofluorescence test (IIF) for *Leishmania*, held in another laboratory was negative. This examination was performed with a kit containing *L. (L.) infantum*, for diagnosis of visceral

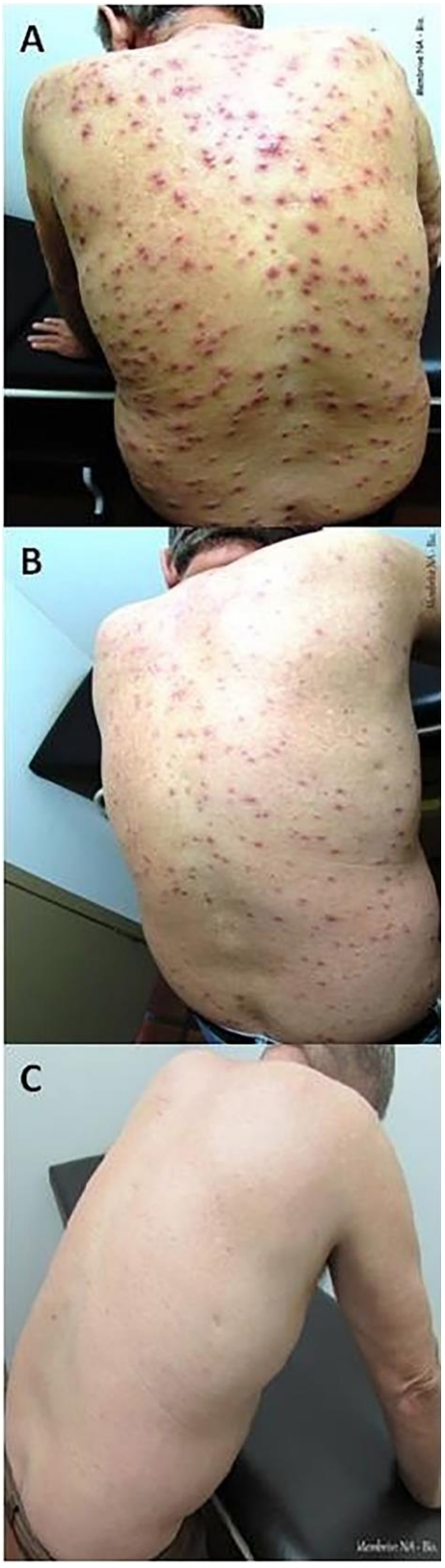

Figure 1 - Patient with disseminated leishmaniasis. (A) Disseminated lesions before treatment; (B) After treatment with 60 ampoules of Glucantime®; (C) After treatment with 120 ampoules of Glucantime®

leishmaniasis. Finally, after 5 months, the patient was sent to an infectious disease specialist of the Laboratory of Medical Entomology from the city of Arapongas, and amastigote forms of *Leishmania* were detected via direct search (DS) of material collected from the primary lesion of the right leg and three other lesions (face, abdomen, and back). Lesion aspirates were mixed with a saline solution containing penicillin G potassium (Sigma) (25,000 IU/mL), and streptomycin (Sigma) (2 mg/mL), at 4 ºC for 24 h; later they were incubated in 199 medium, containing 10% of inactivated fetal bovine serum at 25 °C and the presence of the parasite was detected after five days. It was isolated in the Laboratory of Leishmaniasis from *Universidade Estadual de Maringá* (UEM) and was sent to *Coleção de Leishmania do Instituto Oswaldo Cruz* (CLIOC), Rio de Janeiro, Brazil, for identification. The parasite was identified as *L. (V.) braziliensis* (IOC-L 3636).

After the diagnosis, the patient was treated with intravenous Glucantime® (Sanofi-Aventis Farmacêutica Ltda.), 20 mg/kg /day for 20 days (August 7, 2015, to August 26, 2015). The patient showed a great improvement, with partial healing of lesions, requiring more 20 days of treatment (August 27, 2015, to September 9, 2015), according to the above described procedure. In total, 120 ampoules of glucantime were used. The patient was discharged and showed clinical cure one month after the end of treatment.

DISCUSSION

The extraordinary number of 1,119 lesions observed in the patient is the most important fact in this case. The parasite was identified in the CLIOC as *L. (V.) braziliensis*, confirming that most of the DL cases described in Brazil are associated with this species of *Leishmania*[4,11]. In *Paraná* State, the occurrence of human and canine-CL cases is mainly attributed to infection by *L. (V.) braziliensis*[12].

The current case draws attention to the number of lesions compared to other cases described in Brazil and in the Southern region, given that the cases of CL have been reported almost exclusively in the North and Northeast of Brazil[4,5,11]. In *Maranhão State*, Galvão *et al.*[5] reported a patient with 58 lesions; Carvalho *et al.*[8], in the State of Bahia, found in eight patients, a number of lesions ranging from 75 to 800. Turetz *et al.*[11], also in Bahia, described 42 cases of DL, with the number of lesions ranging from 10 to 300. They drew attention to the increase in the number of patients with DL, classifying it as an emerging form of leishmaniasis, with distinct clinical forms, associated with agricultural activities and the immune response of the host[11,13]. Nevertheless, there is evidence that isolates of *L. (V.) braziliensis*- from DL patients differ genotypically from the ones isolated from patients with cutaneous and mucosal leishmaniasis[14,15], and DL isolates also induce higher inflammatory responses than isolates from CL patients[16].

The dissemination of the lesions in the studied patient occurred in the third month, starting from the beginning of the first lesion. However, there was no impairment of mucous tissue. DL presents numerous acneiform, papular and ulcerated lesions that may arise abruptly, suggesting the hematogenous or lymphatic dissemination of the parasite[11]. Usually, the finding of amastigote forms of *Leishmania* on DS for the diagnosis of DL is low[2,13], but in this case, the number of amastigote forms of *Leishmania* detected on DS was abundant, although the disease had appeared 150 days before being confirmed. The patient's weight loss (15 kg) is not something new, given that fever, general malaise, muscle pain and especially weight loss-are reported among the various systemic manifestations of DL[2,5,11].

Despite biopsy and IIF negative results, the abundant amastigote forms found in the sample collected from lesions and the rapid development in culture medium were decisive factors for the fast and accurate diagnosis of the current DL case. Treatment with meglumine antimoniate was successful, and the response to the medication was quick, given that the patient had the DL diagnosis after five months from the onset of the first lesion. It is noteworthy that the patient did not exhibit any signs of recurrence of -disease until June 2016, nine months after the end of the treatment.

In addition to the extraordinary number of cutaneous lesions, which motivated this case report, the difficult path search for treatment by the patient, who lived in an important endemic area of TL should be pointed. It shows clearly the lack of preparation of the staff working in the Brazilian health system, mainly concerning vector-borne diseases like leishmaniasis.

REFERENCES

1. World Health Organization. Leishmaniasis. Geneva: WHO; 2016. [cited 2017 Apr 12]. Available from: http://www.who.int/leishmaniasis/en/
2. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Guia de vigilância epidemiológica. 7ª ed. Brasília: Ministério da Saúde; 2009.
3. Torres O. A leishmaniasis na Bahia. Arq Bras Med. 1920;7:374-425.
4. Costa JM, Marsden PD, Llanos-Cuentas EA, Netto EM, Carvalho EM, Barral A, et al. Disseminated cutaneous leishmaniasis in a field clinic in Bahia, Brazil: a report of eight cases. J Trop Med Hyg. 1986;89:319–23.

5. Galvão CE, Silva AC, Saldanha AC, Silva CM, Costa MR, Costa JM. Leishmaniose cutânea disseminada produzida por Leishmania viannia braziliensis no Estado do Maranhão - Brasil.. Rev Soc Bras Med Trop. 1993;26:121–3.
6. Silva F. Forma raríssima de leishmaniasis tegumentar. Leishmaniose dérmica não ulcerada em nódulos extensas placas infiltradas e hiperpigmentadas. Reunião Anual de Dermato-Sifilógrafos Brasileiros. Rio de Janeiro; 1946.
7. Convit J, Lapenta P. Sobre um caso de leishmaniasis disseminada. Rev Patol Clin (Caracas). 1946;17:153-8.
8. Carvalho EM, Barral A, Costa JM, Bittencourt A, Marsden P. Clinical and immunological aspects of disseminated cutaneous leishmaniasis. Acta Trop. 1994;56:315-25.
9. Hashiguchi Y, Gomez EL, Kato H, Martini LR, Velez LN, Uezato H. Diffuse and disseminated cutaneous leishmaniasis: clinical cases experienced in Ecuador and a brief review. Trop Med Health. 2016;44:2.
10. Monteiro WM, Neitzke HC, Silveira TG, Lonardoni MV, Teodoro U, Ferreira ME. Pólos de produção de leishmaniose tegumentar americana no norte do Estado do Paraná, Brasil. Cad Saude Publica. 2009;25:1083-92.
11. Turetz ML, Machado PR, Ko AI, Alves F, Bittencourt A, Almeida RP, et al. Disseminated leishmaniasis: a new and emerging form of leishmaniasis observed in northeastern Brazil. J Infect Dis. 2002;186:1829-34.
12. Luz E, Membrive N, Castro EA, Dereure J, Pratlong J, Dedet A, et al. Lutzomyia whitmani (Diptera: Psychodidae) as vector of Leishmania (V.) braziliensis in Paraná State, Southern Brazil. Ann Trop Med Parasitol. 2000;94:623-31.
13. Machado PR, Rosa ME, Costa D, Mignac M, Silva JS, Schriefer A, et al. Reappraisal of the immunopathogenesis of disseminated leishmaniasis: in situ and systemic immune response. Trans R Soc Trop Med Hyg. 2011;105:438-44.
14. Schriefer A, Schriefer AL, Góes-Neto A, Guimarães LH, Carvalho LP, Almeida RP et al. Multiclonal Leishmania braziliensis population structure and its clinical implication in a region of endemicity for american tegumentar leishmaniasis. Infect Immun. 2004;72:508-14.
15. Queiroz A, Sousa R, Heine C, Cardoso M, Guimarães LH, Machado PR, et al. Association between an emerging disseminated form of leishmaniasis and Leishmania (Viannia) braziliensis strain polymorphisms. J Clin Microbiol. 2012;50:4028-34.
16. Leopoldo PT, Machado PR, Almeida RP, Schriefer A, Giudice A, de Jesus AR, et al. Differential effects of antigens from L. braziliensis isolates from disseminated and cutaneous leishmaniasis on in vitro cytokine production. BMC Infect Dis. 2006;6:75.

3. CAPÍTULO III

3.1 CONCLUSÕES

1. Há dificuldades para a compreensão da dinâmica entre agente etiológico e hospedeiros em ambientes onde as mudanças produzidas pelas atividades humanas são constantes.
2. A ausência de flebotomíneos dos *genêros Nyssomyia, Lutzomia e Pintomyia* mostra que tocas de tatus não são os abrigos preferenciais destes insetos. Por outro lado, verifica-se que há estreita convivência de *Brumptomyia brumpti* com o tatu *Dasypus novemcinctus.*
3. Das quatro espécies de roedores capturados, com resultados negativo, três, *Akodon spp., Nectomys* spp*., e Oryzomys* spp. já foram determinadas como hospedeiros de *Leishmania.*
4. Pela primeira vez se constata o predomínio da espécie *Pi. fischeri* em coletas de flebotomíneos no estado do Paraná.
5. Relatado os aspectos clínicos do primeiro caso de LD no Sul do Brasil e o primeiro caso de um paciente com mais de 1.000 lesões na história das leishmanioses.
6. O diagnóstico e tratamento precoce da LC em humanos pode evitar a forma disseminada da doença, LD.

3.2 PERSPECTIVAS FUTURAS

É oportuno lembrar que o modelo de descentralização dos serviços de saúde em vigência no Brasil não tem tido o sucesso no caso de endemias que envolvem vetores. As leishmanioses estão entre as doenças mais negligenciadas do mundo e com dificuldades imensas para o estabelecimento de medidas efetivas de vigilância e controle. Daí o desafio para que os parasitologistas persistam na busca de conhecimentos para que venha a ser organizado um programa de vigilância e controle permanente e efetivo destas zoonoses.