Veterinary Parasitology 190 (2012) 591–594

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Temporal distribution of positive results of tests for detecting *Leishmania* infection in stray dogs of an endemic area of visceral leishmaniasis in the Brazilian tropics: A 13 years survey and association with human disease

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ARTICLE INFO

Article history:
Received 2 May 2012
Received in revised form 18 June 2012
Accepted 19 June 2012

Keywords:
Canine visceral leishmaniasis
Montenegro's skin test
Epidemiology
Leishmania infantum
Leishmania chagasi

ABSTRACT

Human visceral leishmaniasis occurs in periodic waves in endemic areas of Brazil. In this study we followed the prevalence of human visceral leishmaniasis and of *Leishmania infantum* infection in stray dogs of an endemic area of visceral leishmaniasis at periods of time between 1997 and 2010. Prevalence of human visceral leishmaniasis had two peaks (40 cases) in 1997 and 2006 with sharp declines to 2 cases in 2001 and to 5 cases in 2008. Similar fluctuations were also observed in the occurrence of positive spleen culture and anti-*Leishmania* serology in dogs, although the proportion of dogs with active spleen parasitism remained relatively high even in the periods of low prevalence of human disease. These observations support the notion that stray dogs may constitute a renewable source of parasites, capable of sustaining the persistence of the infection in urban areas, even in periods of low transmission by phlebotomines.

Zoonotic visceral leishmaniasis is endemic in the American continent, in the Mediterranean basin and in some non-Mediterranean parts of Asia and Africa. Dogs are the main domestic reservoir of the parasite and a variety of phlebotomines such as *Lutzomyia longipalpis* (in the Americas), *Phlebotomus perniciosus* and *Phlebotomus ariasi* (in the Mediterranean basin) serve as vector of the disease (Desjeux, 2001; Martin-Sanchez et al., 1994). A pattern of occurrence of human visceral leishmaniasis in periodic epidemic waves, spanning many years, has been observed in endemic areas of Brazil (Badaro et al., 1986; Franke et al., 2002; Sherlock, 1996). The reasons for such fluctuation in the incidence of human cases of the disease are poorly understood. However, climatic changes affecting the population dynamics of humans, animal hosts, and sand fly (phlebotomine) vectors has been reputed as a determining factor of the fluctuation in the number of human visceral leishmaniasis cases (Franke et al., 2002; Quinnell and Courtenay, 2009). In spite of this variation in the occurrence of the disease, the fact that periodical outbreaks of

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http://dx.doi.org/10.1016/j.vetpar.2012.06.025

human visceral leishmaniasis are observed in the same endemic area, suggests that the parasite is maintained in the endemic areas, even in periods in which human disease is nearly absent.

In this study we examined the distribution of outbreaks of human visceral leishmaniasis and the prevalence of *Leishmania infantum* (syn *Leishmania chagasi*) infection in dogs of a visceral leishmaniasis endemic area in the period between 1997 and 2010. The data on the tests performed upon the stray dog population was obtained from the records of a collaborative study carried out by the Gonçalo Moniz Research Center, FIOCRUZ, Municipality Zoonosis Control Services of Jequié and the Endemic Diseases Control Center Piraja da Silva – PIEJ (Jequié, BA, Brazil), aiming at different aspects of canine visceral leishmaniasis (Baleeiro et al., 2006; Dos-Santos et al., 2008; Paranhos-Silva et al., 2001). The stray dogs were collected from the streets of Jequié (an endemic area of visceral leishmaniasis in Bahia state, Brazil), and subjected to commonly used tests for detecting infection by *L. infantum*: ELISA, for detecting anti-*Leishmania* specific antibodies in the serum; culture of spleen aspirate for promastigote isolation; and leishmanin (Montenegro's) skin test (LST). The technical details of the anti-*Leishmania* ELISA, the LST and the splenic culture for *Leishmania* isolation have been reported elsewhere (Dos-Santos et al., 2008). Samples of the parasites isolated from the dogs were identified as *L. infantum*. Groups of 38–82 stray dogs were examined in each year of the study (Table 1). The data on human cases and domiciled dog serology were collected from the records of the PIEJ. Human diagnosis of visceral leishmaniasis was based on clinical and laboratorial signs of the disease and a positive ELISA. Estimate of domiciled dog infection was performed by immunofluorescence test using the IFI-leishmaniose canina-Bio-Manguinhos kit (FIOCRUZ, Rio de Janeiro, Brazil), following the manufacturer's instructions.

During the period of the study, the number of human visceral leishmaniasis cases decreased from 40 in 1997 to 2 in 2001 and raised again to 35 cases in 2004, declining to 5 cases in 2008 (Table 1 and Fig. 1). In the same period, fluctuations were also observed in the prevalence of dogs with positive tests for *Leishmania* infection, with a decrease in the proportion of animals with evidence of infection (presenting with a positive ELISA, spleen culture or LST) from 66% in 1997 to 36% in 2001 reaching 87% in 2010 (Table 1). This data on the prevalence of positive tests in stray dogs was deeply influenced by the test used in the study. Nevertheless, even when only the spleen culture, the least sensitive test, is considered, the prevalence of infection in dogs remained high ($31 \pm 11\%$). Even in the period of lowest incidence of human cases (2001), 17% of the stray dogs had positive spleen cultures. These observations support the idea that dogs with active *L. infantum* infection maintain parasites in circulation within local host communities, even in periods of low transmission by phlebotomines. The high levels of active infection detected among the stray dog population may be related to: (1) continuous dog exposition to sandflies even under conditions of low density of this vector; (2) potential dissemination through non-usual vectors such as fleas; (3) direct transmission between dogs

Table 1
Distribution of human visceral leishmaniasis cases and positive cases of *L. infantum* infection in dogs using different laboratory tests.

Year	Stray dogs									Domiciled dogs			Human beings		
	Spleen culture		ELISA			LST		Any test		Immunofluorescence			VL cases		
	Ratio	(%)	[CI]	Ratio	(%)	[CI]	Ratio	(%)	[CI]	[CI]	Ratio	(%)	[CI]	*N*	([a])	[CI]
1997	16/45	(36)	[21–50]	24/47	(51)	[36–66]	4/17	(24)	[1–46]	[52–80]	31/47	(66)	[52–80]	794/16558	(5)	[4.5–5.1]	40	(25)	[17–32]
1998	13/64	(20)	[10–30]	29/81	(36)	[25–46]	15/65	(23)	[13–34]	[36–59]	36/82	(44)	[36–59]	84/529	(16)	[12.8–19.0]	19	(11)	[7–17]
1999	12/34	(35)	[18–52]	11/38	(29)	[14–44]	15/38	(39)	[23–56]	[47–79]	24/38	(63)	[47–79]	218/15291	(1)	[1.2–1.6]	18	(10)	[6–16]
2000	7/39	(18)	[5–31]	26/39	(67)	[51–82]	3/39	(8)	[−1 to 16]	[50–80]	26/40	(65)	[50–80]	241/4463	(5)	[4.7–6.1]	8	(4)	[2–9]
2001	6/35	(17)	[4–30]	7/42	(17)	[5–28]	6/42	(14)	[3–25]	[21–51]	15/42	(36)	[21–51]	51/3357	(2)	[1.1–1.9]	2	(1)	[0–5]
2004	24/48	(50)	[35–65]	31/48	(65)	[51–79]	9/48	(19)	[7–30]	[58–84]	34/48	(71)	[58–84]	256/4063	(6)	[5.6–7.0]	35	(24)	[17–33]
2006	18/45	(40)	[25–55]	28/51	(55)	[41–69]	2/53	(4)	[−2 to 9]	[47–74]	32/53	(60)	[47–74]	945/5437	(17)	[16.4–18.4]	34	(23)	[16–32]
2008	19/53	(36)	[22–49]	43/53	(81)	[70–92]	5/55	(9)	[1–2]	[68–90]	44/56	(79)	[68–90]	505/5309	(10)	[8.7–10.3]	5	(3)	[1–8]
2010	18/58	(31)	[19–43]	50/61	(82)	[72–92]	ND	ND	ND	[78–96]	53/61	(87)	[78–96]	191/1185	(16)	[14.0–18.2]	15	(10)	[6–16]

Ratio, number of animals with positive test/total number of tested animals; CI, 95% confidence interval; LST, leishmanin skin test; VL, visceral leishmaniasis *N*, number of cases.
[a] Number of cases per 100.000 habitants.

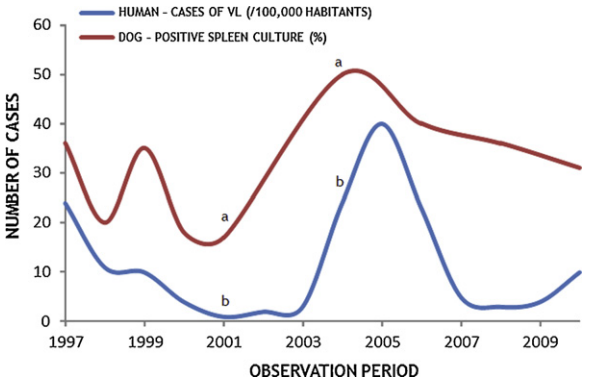

Fig. 1. Frequency of human cases of visceral leishmaniasis and of positive spleen culture for *Leishmania* in dogs from Jequié, BA, Brazil, in the period between 1997 and 2010. The characters a and b indicates time points with significantly different prevalence of positive spleen culture in dogs (a, 95% confidence interval of 4–30 in 2001 and 35–65 in 2004) or human cases of visceral leishmaniasis (b, 95% confidence interval of 0–5 in 2001 and 17–33 in 2004).

through wounds or secretion contacts during fights and sexual intercourse, both usual in the packs of stray dogs; (4) factors, such as malnutrition and co-infections, potentially leading to progression of previously controlled infection in some animals, as observed in other species (Enserink, 2000; Ferreira et al., 2009; Quinnell and Courtenay, 2009). In fact, the results for different tests used to assess *Leishmania* infection in dogs in this study depends upon the stage of infection and susceptibility/resistance of the animals to the development of visceral leishmaniasis (Cardoso et al., 2007; Dos-Santos et al., 2008; Paranhos-Silva et al., 2003; Santana et al., 2008). For instance, discrepancies are expected when the results of the LST, which may reflect some level of resistance to the development of disease, is compared with positive spleen culture which reflects an active infection (Dos-Santos et al., 2008; Santana et al., 2008).

It is noteworthy the similarity between the shape of the curves that represent the frequencies of human cases of visceral leishmaniasis and that of the positivity in culture of dog spleen aspirates (Fig. 1). Parasite detection in internal organs (spleen, liver or bone marrow) is associated with active parasitism and high parasite burden, both associated with disease (Paranhos-Silva et al., 2003; Reis et al., 2006). Hence such similarity in curve shapes of the frequency in human disease and active parasitism in dogs suggest the effect of a possible environmental factor, such as a high density of infected phlebotomines, determining the outburst of disease in both species.

It would be interesting to compare the infection rate between housed and stray dogs in an endemic area. In the time period of this report, however, this was not done in this work, as the infection rates for housed dogs, which were far lower than that for the stray dogs (Table 1), were determined by immunofluorescence and not by ELISA (as was done for the stray dogs). Although it have been shown that immunofluorescence is less sensitive than ELISA in the detection of *Leishmania* infection, it is interesting to notice that even the estimate of stray dog infection based on spleen culture, which is considered less sensitive than the immunofluorescence test, showed a high proportion of infection among stray dogs in the whole period of the study. One cannot, therefore, exclude the possibility that, in comparison to housed dogs, stray dogs are more exposed to infection and to factors that determine the emergence of visceral leishmaniasis and/or positive spleen or skin parasitisms. In this case, such a population of stray dogs, frequent in most endemic areas of Brazil, may constitute a renewable source of parasites, capable of sustaining the persistence of the infection in urban areas, even in periods of low transmission by phlebotomines.

Ethics statement

All procedures involving animals were conducted in accordance with Brazilian Federal Law on Animal Experimentation (Law 11794) (http://www.planalto.gov.br/ccivil_03/_ato20072010/2008/lei/l11794.htm), with the Oswaldo Cruz Foundation guidelines for research with animals (http://sistemas.cpqam.fiocruz.br/ceua/hiceuaw000.aspx) and with the manual for the surveillance and control of visceral leishmaniasis. This study was approved by the institutional ethics committee for the use of animals in research (CPqGM-FIOCRUZ, CEUA, license No. 040/2005).

Acknowledgements

We thank Dr. Maria Conceição Chagas for her assistance in the statistical analysis and representation of the data presented in the manuscript. This work was supported by the Fundação Oswaldo Cruz grant PAPES-V 403470/2008-0, FAPESB grant APR0434/2005 and the Brazilian National Research Council – CNPq grant 302096/2009-4.

References

Badaro, R., Jones, T.C., Lorenco, R., Cerf, B.J., Sampaio, D., Carvalho, E.M., Rocha, H., Teixeira, R., Johnson Jr., W.D., 1986. A prospective study of visceral leishmaniasis in an endemic area of Brazil. J. Infect. Dis. 154, 639–649.

Baleeiro, C.O., Paranhos-Silva, M., Dos Santos, J.C., Oliveira, G.G., Nascimento, E.G., de Carvalho, L.P., Dos-Santos, W.L., 2006. Montenegro's skin reactions and antibodies against different *Leishmania* species in dogs from a visceral leishmaniosis endemic area. Vet. Parasitol. 139, 21–28.

Cardoso, L., Schallig, H.D., Cordeiro-da-Silva, A., Cabral, M., Alunda, J.M., Rodrigues, M., 2007. Anti-*Leishmania* humoral and cellular immune responses in naturally infected symptomatic and asymptomatic dogs. Vet. Immunol. Immunopathol. 117, 35–41.

Desjeux, P., 2001. The increase in risk factors for leishmaniasis worldwide. Trans. R. Soc. Trop. Med. Hyg. 95, 239–243.

Dos-Santos, W.L., Jesus, E.E., Paranhos-Silva, M., Pereira, A.M., Santos, J.C., Baleeiro, C.O., Nascimento, E.G., Moreira, E.D., Oliveira, G.G., Pontes-de-Carvalho, L.C., 2008. Associations among immunological, parasitological and clinical parameters in canine visceral leishmaniasis: emaciation, spleen parasitism, specific antibodies and leishmanin skin test reaction. Vet. Immunol. Immunopathol. 123, 251–259.

Enserink, M., 2000. Infectious diseases Has leishmaniasis become endemic in the U.S.? Science 290, 1881–1883.

Ferreira, M.G., Fattori, K.R., Souza, F., Lima, V.M., 2009. Potential role for dog fleas in the cycle of *Leishmania* spp. Vet. Parasitol. 165, 150–154.

Franke, C.R., Ziller, M., Staubach, C., Latif, M., 2002. Impact of the El Nino/Southern Oscillation on visceral leishmaniasis, Brazil. Emerg. Infect. Dis. 8, 914–917.

Martin-Sanchez, J., Guilvard, E., Acedo-Sanchez, C., Wolf-Echeverri, M., Sanchiz-Marin, M.C., Morillas-Marquez, F., 1994. *Phlebotomus perniciosus* Newstead, 1911, infection by various zymodemes of the *Leishmania infantum* complex in the Granada province (southern Spain). Int. J. Parasitol. 24, 405–408.

Paranhos-Silva, M., Oliveira, G.G., Reis, E.A., de Menezes, R.M., Fernandes, O., Sherlock, I., Gomes, R.B., Pontes-de-Carvalho, L.C., dos-Santos, W.L., 2003. A follow-up of Beagle dogs intradermally infected with *Leishmania chagasi* in the presence or absence of sand fly saliva. Vet. Parasitol. 114, 97–111.

Paranhos-Silva, M., Pontes-de-Carvalho, L.C., de Sa Oliveira, G.G., Nascimento, E.G., dos-Santos, W.L., 2001. Skin reactions to thimerosal and *Leishmania* in dogs from a leishmaniasis endemic area: it is better to keep them apart. Mem. Inst. Oswaldo Cruz 96, 679–681.

Quinnell, R.J., Courtenay, O., 2009. Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. Parasitology 136, 1915–1934.

Reis, A.B., Teixeira-Carvalho, A., Vale, A.M., Marques, M.J., Giunchetti, R.C., Mayrink, W., Guerra, L.L., Andrade, R.A., Correa-Oliveira, R., Martins-Filho, O.A., 2006. Isotype patterns of immunoglobulins: hallmarks for clinical status and tissue parasite density in Brazilian dogs naturally infected by *Leishmania* (*Leishmania*) *chagasi*. Vet. Immunol. Immunopathol. 112, 102–116.

Santana, C.C., Vassallo, J., de Freitas, L.A., Oliveira, G.G., Pontes-de-Carvalho, L.C., dos-Santos, W.L., 2008. Inflammation and structural changes of splenic lymphoid tissue in visceral leishmaniasis: a study on naturally infected dogs. Parasite Immunol. 30, 515–524.

Sherlock, I.A., 1996. Ecological interactions of visceral leishmaniasis in the state of Bahia, Brazil. Mem. Inst. Oswaldo Cruz 91, 671–683.