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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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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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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]	Ratio	(%)	[CI]	*N*	([a])	[CI]
1997	16/45	(36)	[21–50]	24/47	(51)	[36–66]	4/17	(24)	[1–46]	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]	36/82	(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]	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]	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]	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]	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]	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]	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	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.