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Short communication

Detection and quantification of *Leishmania braziliensis* in ectoparasites from dogs

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ABSTRACT

American cutaneous leishmaniosis (ACL) is a disease caused by different species of *Leishmania* protozoa, *Leishmania braziliensis* being the main species found in Brail. In this study, two rural areas in Pernambuco, northeastern Brazil, where ACL is endemic, were selected. Genomic DNA was extracted from canine ectoparasites (ticks, fleas, and lice) and tested using a conventional PCR and a quantitative real time PCR. A total of 117 ectoparasites were collected, being 50 (42.74%) of them positive for *L. braziliensis* (in at least one PCR protocol), with a mean parasite load of 14.14 fg/μL. Furthermore, 46 (92.00%) positive ectoparasites were collected from positive dogs and 4 (8.00%) from negative ones. This study reports the detection of *L. braziliensis* DNA in ectoparasites, but does not prove their vector competence. Certainly, experimental transmission studies are necessary to assess their role, if any, in the transmission of *Leishmania* parasites to dogs.

1. Introduction

American cutaneous leishmaniosis (ACL) constitutes an important public health problem, whose control is difficult to achieve. In Brazil, ACL is on the rise, with autochthonous cases of human infection by *Leishmania braziliensis* in all Brazilian regions (Brito et al., 2012). The difficulties in controlling ACL may be related to the complex epidemiology of this disease, which involves the participation of different vector species, different wild and domestic animal hosts (small rodents, marsupials, dogs, cats, and horses) and humans as well, that interact in different kinds of environments (e.g., primary forests, human houses, and crop plantations).

In spite of the numerous reports of natural infection by *L. braziliensis* in dogs, their role in the life cycle of this parasite has not been clarified yet (Brandão-Filho et al., 2011). In recent years, there has been a growing scientific interest about the possible role of ectoparasites in the epidemiology of canine visceral leishmaniosis (Coutinho et al., 2005; Solano-Gallego et al., 2012), but their actual importance, if any, in the transmission cycle of *Leishmania infantum* remains unknown (Dantas-Torres, 2011).

In the present study, we assessed the presence of *L. braziliensis* in canine ectoparasites collected in the framework of a previous study (Morais et al., 2013) carried out in northeastern Brazil.

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2. Material and methods

Ectoparasites were collected from dogs living in two rural areas in Pernambuco State. Site A and site B were in the municipalities of Igarassu (7°50′02″ S, 34°54′21″ W) and Passira (07°59′42″ S, 35°34′51″ W), respectively. Ectoparasites were collected from dogs by hand collection and maintained in 70% ethanol until identification using morphological taxonomic keys (Aragão and Fonseca, 1961; Guimarães et al., 2001). The Commission for Ethics in the Use of Animals (CEUA, FIOCRUZ, Brazil) of our institution approved the study protocol and owners gave their informed written consent before ectoparasite collection.

DNA from ectoparasites was extracted using Illustra tissue and cells genomicPrep Mini Spin kit (GE Healthcare), following the manufacturer's instructions. The extracted DNA was stored at −20 °C for subsequent amplification by a conventional polymerase chain reaction (PCR) according to Brujin and Barker (1992) and by a quantitative real time PCR (qPCR), as described elsewhere (Paiva-Cavalcanti et al., in press). The level of agreement between results obtained with both PCR protocols was assessed using Kappa statistics. Statistical analysis was performed using BioEstat 5.0 (Ong Mamiraua. Belém, PA, Brazil).

3. Results

A total of 117 ectoparasites (21 ectoparasites from site A and 96 from site B) were collected from 99 dogs. Ectoparasites included fleas (*Ctenocephalides felis*), ticks (*Rhipicephalus sanguineus*), and lice (*Heterodoxus spiniger*).

A total of 50 (42.74%) ectoparasites were positive for *L. braziliensis* (Fig. 1), being 12 (41.38%) *C. felis*, 32 (42.67%) *R. sanguineus*, and 6 (46.15%) *H. spiniger* (Table 1). The mean parasite load detected in positive ectoparasites was 14.14 fg, ranging from 0.37 fg to 113.37 fg (Ct 38.60 and Ct 31.38, respectively). Furthermore, a total of 46 (92.00%) positive ectoparasites were collected from positive dogs and four from negative ones (Table 2).

Comparing the two PCR protocols used (i.e., PCR and qPCR), a low level of agreement was found (Table 1), being most qPCR-positive ectoparasites negative when tested by PCR.

4. Discussion

Again, this study reports the detection and quantification of *L. braziliensis* DNA in ectoparasites collected from dogs living in an area of ACL-endemicity. Remarkably, some positive ectoparasites were collected from negative dogs, indicating that they acquired the infection by feeding on another infected dog. Dogs living in the study sites are highly exposed to *L. infantum* and *L. braziliensis* (Morais et al., 2013; Paiva-Cavalcanti et al., in press) and an outbreak of ACL in humans has recently been reported in site A (data not shown). Interestingly, human cases of ACL have not been reported in site B, where visceral leishmaniosis is endemic. This suggests that human cases of ACL may have been underreported in this area. On the other hand, it is important to clarify that the presence of dogs and ectoparasites positive to *L. braziliensis* in site A, does not imply that dogs or ectoparasites are involved in transmission cycle of this parasite.

Domestic animals are not considered important reservoirs of *L. braziliensis* (Brandão-Filho et al., 2011). However, some of these animals (e.g., chickens, cattle, and horses) are highly attractive to phlebotomine sand fly vectors and may play a part in the epidemiology of ACL (Brandão-Filho et al., 2011). The construction of human houses and the presence

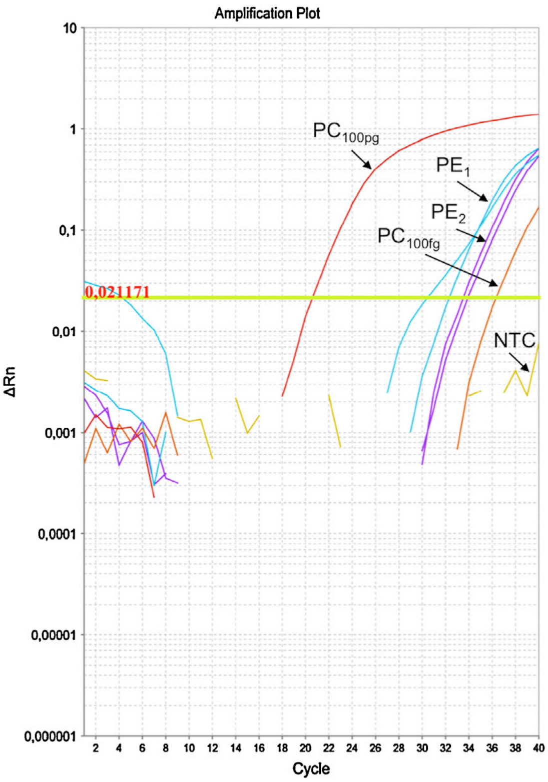

Fig. 1. Real time PCR amplification curve showing *L. braziliensis*-positive ectoparasites (PE_1 and PE_2) collected from dogs. Positive controls (100 pg/μL and 100 fg/μL = $PC_{100\,pg}$ and $PC_{100\,fg}$, respectively) and no-template control (NTC) are also shown.

Table 1
Positivity to *L. braziliensis* by conventional PCR (PCR) and real-time PCR (qPCR) in ectoparasites collected from dogs in Pernambuco, northeastern Brazil.

Ectoparasites	*n*	Positive (overall)		PCR	qPCR	Kappa statistics	
		n	%			Kappa	*P* value
Ctenocephalides felis	29	12	41.38	2	11	0.04	0.36
Heterodoxus spiniger	13	6	46.15	1	5	−0.15	0.21
Rhipicephalus sanguineus	75	32	42.67	1	31	−0.03	0.20
Total	117	50	42.73	4	47	−0.03	0.26

Table 2
Positivity to *L. braziliensis* in ectoparasites collected from dogs either positive or negative by conventional PCR and/or real-time PCR.

Ectoparasites	*n* total	From positive dogs (*n* = 252)		From negative dogs (*n* = 23)	
		n	%	*n*	%
Ctenocephalides felis	12	10	83.33	2	16.67
Heterodoxus spiniger	6	5	83.33	1	16.67
Rhipicephalus sanguineus	32	31	96.87	1	3.13
Total	50	46	92.00	4	8.00

of domestic animals near forested areas, as commonly observed in rural areas in northeastern Brazil, may affect the epidemiology of ACL. Indeed, large populations of phlebotomine sand flies may establish in animal shelters, such as chicken coops and stables (Guimarães et al., 2012), thus increasing the risk of human infection (Brandão-Filho et al., 1999). Deforestation, changes in land use, construction of roads and climate changes may also alter the epidemiology of ACL, as these factors may directly impact on the ecology and distribution of vectors and reservoirs of *Leishmania* parasites (Dantas-Torres et al., 2012). However, it is imperative that populations in endemic areas are informed by public health authorities that dogs are not considered epidemiologically relevant reservoirs of *L. braziliensis* to avoid the unnecessary elimination of infected dogs.

Recent studies have reported the detection of *L. infantum* DNA or RNA fragments in ticks, fleas and lice (Coutinho et al., 2005; Colombo et al., 2011; Solano-Gallego et al., 2012; Morais et al., 2013). In this study, we reported for the first time the detection of *L. braziliensis* in ticks, fleas and lice collected from positive and negative dogs. Interestingly, most *L. braziliensis*-positive ectoparasites were also *L. infantum*-positive (data not shown). Furthermore, the mean parasite load found for *L. braziliensis* (14.14 fg/μL) was higher than to that previously reported for *L. infantum* (9.10 fg/μL) (Morais et al., 2013). Certainly, these findings should be interpreted with caution because blood-feeding arthropods may ingest whatever is in the blood of their hosts (Otranto and Dantas-Torres, 2010). This study reports the detection of *L. braziliensis* DNA in ectoparasites, but does not prove their vector competence. Certainly, the finding of *L. braziliensis*-positive chewing lice (*H. spiniger*) may indicate that even non-hematophagous arthropods may eventually ingest parasites that are present in the blood or lymph of dogs. However, experimental studies are necessary to achieve a definitive conclusion regarding the role of ectoparasites in the transmission of *Leishmania* parasites to dogs.

Conflict of interest

The authors declare there are no conflicts of interest.

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