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Antibodies against *Lutzomyia longipalpis* saliva in the fox *Cerdocyon thous* and the sylvatic cycle of *Leishmania chagasi*

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Summary Sera of 11 wild *Cerdocyon thous* foxes from an endemic area for American visceral leishmaniasis were tested for the presence of antibodies against salivary gland homogenates (SGH) of *Lutzomyia longipalpis*. All foxes had higher levels of anti-*Lu. longipalpis* SGH antibodies than foxes from non-endemic areas, suggesting contact between foxes and the vector of visceral leishmaniasis. Sera of humans and dogs living in the same area were also tested for reactivity against *Lu. longipalpis* SGHs and had a lower proportion of reactivity than foxes. Antibodies against *Leishmania chagasi* were not detected in any of the foxes, but three foxes showed the presence of parasites in the bone marrow by direct examination, PCR or by infecting the vector. Both humans and dogs had higher levels of anti-*Le. chagasi* IgG antibodies than *C. thous*. The finding of an antibody response against saliva of *Lu. longipalpis* among *C. thous* together with the broad distribution of the vector in resting areas of infected foxes suggests that the natural foci of transmission of *Le. chagasi* exists independently of the transmission among dogs and humans.

1. Introduction

American visceral leishmaniasis (AVL) is an important vector-borne human disease caused by the protozoa *Leishmania chagasi*. More common in northeast Brazil but expanding to the Amazon rain forest and to larger cities of the industrialised southeast, the disease kills 5—10% of over 4000

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persons that become sick every year in this country. Patients almost uniformly present with fever, anaemia and hepatosplenomegaly. The main complications are opportunistic infections or severe bleeding (Jerônimo et al., 1994; Werneck et al., 2003).

In the New World, AVL is transmitted by the bite of *Lutzomyia longipalpis* after this sandfly has bitten an infected vertebrate host. Female sandflies inject saliva when probing for a blood meal (Ribeiro et al., 1986). The saliva contains a number of substances that are able to interfere with vertebrate haemostatic and inflammatory responses (Charlab et al., 1999; Titus and Ribeiro, 1990). It also triggers an immune response, which, as also happens upon exposure to saliva of other blood-feeding arthropods, leads to the production of specific antibodies. This response against salivary proteins has already been used as an epidemiological marker of exposure to vectors (Barral et al., 2000; Brummer-Korvenkontio et al., 1994; Feingold, 1968; Wikel, 1996).

Humans, dogs, wild canines and opossums are naturally infected with *Le. chagasi* (Deane and Deane, 1954a; Pearson et al., 1999; Sherlock et al., 1984). However, dogs are thought to be the most important source of infection for humans because they easily transmit *Le. chagasi* to the sandfly vector, they present a higher proportion of natural infection than humans (Evans et al., 1992) and they are more abundant and live closer to humans than wild animals (Costa, 1997). On the other hand, the early finding of infected *Pseudalopex vetulus* (= *Lycalopex vetulus*) foxes (Deane and Deane, 1954b) in northeastern Brazil and recently of the more abundant species of fox *Cerdocyon thous* also infected with *Le. chagasi* in the Amazon area (Lainson et al., 1969) suggests the existence of a sylvatic cycle that may have evolved before Europeans arrived to the New World (Lainson et al., 1987; Momem et al., 1987). However, sylvatic transmission of *Le. chagasi* has never been definitely demonstrated.

This issue has practical consequences. Since dogs are thought to be the main vertebrate reservoir, Brazil developed a huge programme of culling infected dogs (Lacerda, 1994). If transmission originates solely from dogs, reservoir control might be more easily achieved and eradication could be envisioned. However, if a wild cycle does exist, transmission from sylvatic animals to humans and dogs could dampen the effect of reservoir control measures.

Finding animals with antibodies specific for saliva of *Lu. longipalpis* would therefore indicate natural contact of the vertebrate host with the sandfly vector of AVL and suggest that both foxes and sandflies occupy the same environment. If so, together they could maintain a putative sylvatic cycle of transmission of *Le. chagasi*. Having captured many foxes in the surroundings of a city where AVL is highly endemic, we decided to verify the possibility of contact of foxes with the abundant local population of *Lu. longipalpis* and to compare the evidence of vector–fox contact with that obtained for contact of local dogs and humans with *Lu. longipalpis*.

2. Materials and methods

2.1. Study area and subjects

Data were collected from the years 2001–2003 in the environs of Teresina, Piauí, situated in middle–northern Brazil. Part of the study was undertaken at the Zoo Botanic Park, located approximately 500 m from the closest neighbourhood but still within the city limits. Most other data were collected approximately 6 km outside the city (Árvore Verde).

A total of 11 *C. thous* foxes were captured in both areas. Two were cubs and five were males. All but one adult female were healthy. The animals were weighed, a veterinarian evaluated the general health condition, and blood and bone marrow samples were collected from all of them. Ten to 20 female *Lu. longipalpis* were allowed to feed in the ear for 20–30 min. A radio-emitting collar was used to track the animals, allowing two more adults and one cub that had recently died to be found. The ethical committee of the Instituto de Doenças Tropicais Natan Portella approved the protocol for human evaluation and informed consent was obtained from each participant. The national institution for protection of the environment (IBAMA) authorised tagging, blood collection and bone marrow aspiration of the animals.

2.2. Bone marrow examination and PCR

Direct examination and culturing in NNN media of bone marrow from each fox was performed. Then, Giemsa-stained slides were scratched and DNA was extracted with phenol–chloroform. Using PCR, a 120 bp template of the constant (generic) region of kDNA was amplified using the primers 13A and 13B, as previously described (Rodgers et al., 1990).

2.3. Sera samples from foxes and controls

Sera were obtained from all 11 *C. thous* captured in Teresina. Eleven samples of *C. thous* were also studied from the non-endemic area of Pelotas, Rio Grande do Sul, at the extreme south of Brazil where *Lu. longipalpis* has never been found. Sera from 59 dogs from the study area and 8 dogs from a non-endemic area (Salvador, Brazil) previously tested as negative to sandfly saliva by ELISA were used as negative controls. The sera of 119 individuals from the study area were also analysed as well as 15 sera of persons from a non-endemic area for AVL in southern Bahia where *Lu. longipalpis* is not present.

Lutzomyia longipalpis comprises over 90% of sandflies captured in the woodlands where the foxes of Teresina area were found (Soares et al., 2004a, 2004b). The other species identified in the area were *Lu. lenti*, *Lu. goiana*, *Lu. evandroi* and *Lu. whitmani*. We evaluated the antibody response to salivary gland homogenates (SGH) of *Lu. longipalpis* and *Lu. whitmani* only, since we do not have established colonies of *Lu. lenti*, *Lu. goiana* or *Lu. evandroi*. Additionally, the serological response to *Lu. intermedia* SGH was also tested. Sera from BALB/c mice bitten by *Lu. whitmani* were used as positive controls to measure the antibody response against the SGH of this species of sandfly.

2.4. Sandflies and preparation of salivary glands

Lutzomyia longipalpis Cavunge strain, *Lu. whitmani* and *Lu. intermedia* were regularly reared. The strain of *Lu. longipalpis* comes from an isolate from an area ~150 km from Salvador and approximately 1000 km apart from Teresina. Adult sandflies were offered cotton containing a sucrose