Can domestic cats be considered reservoir hosts of zoonotic leishmaniasis?

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Canine and human zoonotic leishmaniasis caused by *Leishmania infantum*, which is transmitted by the bite of infected phlebotomine sand flies, is a serious public health problem in the Mediterranean basin and Latin America. Among reports on newly identified mammalian hosts recurrently found infected with *L. infantum*, those regarding domestic cats deserve attention for the potential implications to public health. It has been shown that these animals cohabiting with humans can be infected (although only a few cases develop disease) and harbor parasites in an available way for transmission to competent vectors. Nonetheless, their role as reservoir hosts is still controversial.

Dogs as reservoir hosts of zoonotic leishmaniasis due to *Leishmania infantum*

Dogs have been universally considered the major hosts of *Leishmania infantum* and the main domestic and peridomestic reservoir hosts in the zoonotic cycle of visceral leishmaniasis. This last assumption has been based on the high prevalence of infection in those animals living in endemic areas, with a large proportion of them not showing clinical signs. In addition, it has been proven that phlebotomine vectors of the parasite in the Old and New Worlds, *Phlebotomus* spp. and *Lutzomyia* spp., frequently feed on these animals [1,2]. Several xenodiagnosis studies performed on seropositive dogs (with and without clinical signs) in both Europe and South America showed that a high percentage of these infected animals were infectious to laboratory-reared sand flies [3]. In summary, dogs are reservoir hosts of *L. infantum* because: (i) infection is frequent in the canine population; (ii) infection is normally long-lived but without clinical signs; (iii) they present sufficient parasite loads in the skin and blood increasing the possibility of transmission; (iv) they have a close relationship with humans, and parasites isolated from them are indistinguishable from those of humans; (v) the main vectors feed frequently on them; and (vi) they are infectious to the vector [1,3,4].

Early detection of infected dogs, their close surveillance and treatment are essential control strategies to avoid the spread of canine infection and consequently human zoonotic leishmaniasis (ZL) by blocking parasite transmission to sand flies [5]. Another method focusing on the reservoir to control ZL recommended by the World Health Organization is the culling of infected dogs [4]. Despite that a decrease in incidence of infection was verified in some endemic areas in both dogs and humans following culling of seropositive animals, the efficiency and acceptability of this control strategy is increasingly being debated [3,6]. Furthermore, the implementation of this control strategy in countries where dogs are considered part of the family would not be easily carried out. One of the reasons why this control measure did not prevent or decrease *Leishmania* transmission as expected was probably due to the low coverage of culling, owing to logistic and operational problems, thus leading to a low percentage of infected animals that were eliminated and to the replacement of culled dogs by uninfected susceptible ones. Another hypothesis for the failing of leishmaniasis control when the target to block transmission was the dog could have been related to the existence of reservoir hosts other than dogs which are responsible for the maintenance of the parasite in the domestic and peridomestic habitat. For this reason and because the number of feline leishmaniasis cases reported in previous years has increased, it is necessary to evaluate from an epidemiological and control perspective the real importance of cats in the *Leishmania* domestic cycle.

What defines a reservoir as a host of infection?

Definitions of the term reservoir host with different levels of complexity which are often contradictory have been proposed by various authors [7–9]. The maintenance of many causative agents of disease depends on the presence of several host species [7]. However, when more than one

Glossary

Accidental or incidental host: a host that although infected plays no role in the maintenance of the ecological system [3,4,9].
Ecological system: an environment composed of one or a small number of sand fly vector species and one or a few vertebrate reservoir host species where the parasite is maintained indefinitely [4].
Host: a vertebrate harboring the parasite.
Primary reservoir host: a host that is responsible for maintaining the parasite indefinitely in nature. In these hosts infection is normally without clinical signs [3,4,9].
Secondary reservoir host or minor host: a host that can transmit infection but cannot maintain parasite transmission in the absence of the primary host(s) [3,4,9].

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host species can be infected they are often divided on epidemiological grounds into primary, secondary (or minor) and accidental (or incidental) reservoir hosts (see Glossary). Furthermore, it has been suggested that for a parasite species to persist indefinitely or for a long period of time in a reservoir host population, it must have a basic reproduction number (R_0 = average number of secondary cases arising from a primary case in a susceptible population) ≥ 1 [3]. Using this mathematical model for *L. infantum* transmission in infected dogs it was estimated a $R_0 = 11$ and $R_0 = 9$ in Malta and the Amazon, Brazil, respectively, highlighting the difficulty in control of leishmaniasis in those areas [3]. Existence of two distinct primary reservoir hosts is also possible when peridomestic and sylvatic transmission cycles operate concurrently and are linked via a common vector responsible for the transmission of the parasite between both cycles. This phenomenon was observed in some endemic areas of *L. infantum* infection in the Old World, having the dog as the domestic/peridomestic reservoir host and the red fox (*Vulpes vulpes*) as the sylvatic one. However, it is difficult to differentiate between primary or secondary reservoir hosts, when animal species incriminated in the transmission of the pathogen share the same geographic area, as occurs with *L. infantum*, dogs and cats.

Are domestic cats reservoir hosts of *Leishmania infantum?*

Leishmaniasis in cats (*Felis catus*) was first described in 1912 in Algeria, from a household where a dog and a child were also infected [10]. More recently, *L. infantum* infection in domestic cats has been reported in several endemic countries from Europe, the Middle East and Brazil [10–23]. Thus, an increasing trend with regard to cats as a potential domestic reservoir host of *L. infantum* exists because they are: (i) naturally susceptible to infection by *L. infantum*, normally without development of clinical signs (these, when present, are often cutaneous but systemic involvement has also been recorded); (ii) a blood source for some *Leishmania* vectors (*Phlebotomus perniciosus* and *Lutzomyia* spp.); (iii) harboring parasites in an available way to infect the vector; and (iv) among the most popular pet animals around the world, often present in areas where the peridomestic and domestic transmission cycles of the parasite occur [23–26]. Despite this evidence, the epidemiological importance of cats in leishmaniasis is still controversial; on the one hand, they are often recognized as accidental hosts, whereas on the other hand, they are considered as secondary or alternative reservoir hosts to the dogs [13–17]. Even for those who defend domestic cats as important for the maintenance of the *L. infantum* life cycle, the categorization of these animals as primary or secondary reservoir hosts is still unclear. Thus, from an epidemiological and control perspective it would be very important to evaluate the proportion of transmission in endemic areas attributable to cats to clarify if these animals are reservoir hosts sustaining and spreading ZL. Thus, extensive feline surveys, out of transmission season, should be performed in a representative sample using distinct methodologies to obtain an accurate prevalence. Simultaneously, prevalence of infection in dogs living in the same place as cats should be determined at the same time and using the same methods to evaluate the relative importance of these two animal species in the epidemiology of *Leishmania* infection. The dependence level and the direction of parasite transmission (i.e. if cats are inoculated with *L. infantum* through the bite of competent vectors that become infected after feeding on dogs harboring parasites or vice versa) between these animal species are also important issues.

Which indicators can help identify cats as reservoir hosts of *Leishmania infantum*?

Identification of natural infection, which can be determined through antibody detection or isolation of the infectious agent or its genes from the host, is one of the criteria that must be met for an infected host to be defined as a reservoir host [8]. Several feline serological surveys have been made to detect anti-*Leishmania* antibodies showing a range from 0.6% to 59% in different geographic areas [11,13–17,21,22,27–30]. Nevertheless, in most reports the specific antibodies were absent or low suggesting that serology is probably not the best method of diagnosis. This absence or low level of antibodies could be related to the fact that cats do not suffer from disequilibrium of the immune status which would lead to overproduction of antibodies as it occurs in dogs. Certainly, it is important to keep in mind that the presence of specific antibodies only indicates that the animal was exposed to infection. Furthermore, because cats have been found naturally infected by *L. amazonensis* [31] and *L. braziliensis* [32] in Brazil, it is important to keep in mind that in areas where *Leishmania* species occur sympatrically, serological crossreactions can interfere in the evaluation about the role of cats as reservoir hosts of *L. infantum*.

The isolation of *Leishmania* and/or its DNA from infected tissues presents better evidence that a species can act as a reservoir host. Prevalence of *Leishmania* infection in cats determined using parasitological methods such as parasite culture, direct microscopy, DNA amplification, histopathological and immunohistochemical identification of *Leishmania* amastigotes in mononuclear phagocyte cells from lymphoid organs, skin and peripheral blood ranged from 0.43% to 61% [10,11,13,15,18,19,22,23,27,33]. These results show that the parasite can colonize cats living in endemic areas. Numerous canine surveys were performed in endemic areas of ZL in the Old and New Worlds to determine prevalence of *L. infantum* infection. Although the detection of specific anti-*Leishmania* antibodies was the methodology used in most of the studies, the infection rate observed using parasitological methods ranged from 15.8% to 97.3% [2,34,35]. In spite of results from canine and feline surveys that showed a higher prevalence of infection in dogs, most parasitological studies in cats only checked the peripheral blood. Nevertheless, the determination of infection at the same time in both animal species living in the same endemic area and with the same laboratorial method has only been evaluated in one recent study [15]. In this survey, 31.4% (43/137) of blood samples from dogs and 18.5% (17/92) from cats collected outside of the transmission period were PCR positive, whereas during the transmission period, *L. infantum* DNA was amplified in 60.0% (9/15) and 22.0% (11/50) in canine and feline samples, respectively. These results indicate that more extensive and well-designed surveys should be done on cats.

Evidence for the presence of *Leishmania* parasites in the peripheral blood has only been provided by the detection of DNA sequences by PCR. Indeed, blood is not a tissue of choice for diagnosing *Leishmania* infection, as amastigotes are hardly ever observed in the blood circulation. This statement was demonstrated by the lower frequency of positive results in the blood of dogs when compared to other tissues/organs [5,15]. Despite that a positive blood–PCR result observed during *Leishmania* transmission season due to natural contamination or transient infection cannot be ruled out, the PCR positivity obtained out of the sand fly period indicates a 'true' infection [10,11,15].

Another criterion to determine if a species can act as a *Leishmania* reservoir host is that parasites isolated from the animal must be biochemically and molecularly indistinguishable from those of humans. Two strains of parasites (one isolated from a popliteal lymph node aspirate and the other one from a bone marrow biopsy performed in cats living in Italy and France, respectively) were identified as *L. infantum* zymodeme MON-1 by isoenzymatic typing at the Centre Nacional de Reference sur les Leishmanioses (Montpellier, France) [36,37]. This zymodeme is responsible for most of the cases of canine and human visceral leishmaniasis around the Mediterranean basin, and to date the only one isolated from infected cats. Furthermore, genotyping of parasite DNA isolated from different tissues of cats (namely blood, popliteal lymph node, skin, bone marrow, liver, spleen and kidneys) living in endemic areas of Brazil, Italy, Iran and Portugal has already been performed showing that all parasites were identified as *L. infantum* [10,15,18,19,23,37,38].

It has been suggested that a criterion for a 'good' reservoir host is that infection should present a chronic evolution, preferably life-long, allowing the animal to survive at least until the next transmission season [10]. In dogs, it is widely accepted that an effective control of host infection and survival is related to the development of a strong cellular immune response to the parasite. These animals are considered resistant to disease and thus present subclinical infection for long periods of time [5]. However, there is no study so far demonstrating feline cellular immune response to *Leishmania*. Therefore, it is essential to evaluate the immune response of cats to infection (with simultaneous analysis of both humoral and cellular immune responses) to determine if the reason why these animals normally do not develop clinical signs or high antibody levels are due to an equilibrium between their immune system and the presence of the parasite or if they can control infection through parasite clearance. Thus, longitudinal studies in naturally infected cats should be performed for a long period of time, for more than one transmission season, to determine if *Leishmania* infection in these animals persists. Nevertheless, apart from maintaining the parasite, the confirmation of the role of such animals as reservoir hosts of *Leishmania* species depends on their infectiousness to the vector. However, the information about this crucial requisite is still scarce. To date, there are only two reports, one from Italy [37] and another from Brazil [38], regarding cats as potential sources of *L. infantum* infection for phlebotomine sand flies. In those experiments, it was demonstrated that two cats chronically infected and submitted to xenodiagnosis were infectious to laboratory-bred *P. perniciosus* and *L. longipalpis*, demonstrating that feeding and infection rates were similar to those obtained with sick dogs submitted to the same conditions. Notably, there is no data regarding the infectiousness of cats to vectors from nature and in feline populations to confirm their role in the zoonotic cycle of leishmaniasis.

Box 1. Outstanding questions: are cats capable of sustaining and spreading *L. infantum* infection?

- Is the proportion of cats infected with *L. infantum* high enough to maintain infection in endemic areas of ZL?
- Do they harbor parasites, in a sufficient number and for a long period of time, in tissues that are accessible to competent vectors?
- How frequently are phlebotomine vector species that share the same habitat as cats and feed on them found naturally infected with *L. infantum*?

The use of topical insecticide treatment of dogs has been shown to be effective in reducing the incidence of canine and human visceral leishmaniasis [3,4,39]. If it is proven that cats are infectious to competent vectors of *L. infantum* at a population level, a major limitation to control infection in these animals would be their sensitivity to pyrethroids. Therefore, another crucial issue that should be addressed in the future is how infection in cats can be prevented because there are no repellents against sand flies that can be used on these animals.

Concluding remarks

In summary, there is much evidence that domestic cats from endemic areas of ZL are often infected with *L. infantum*. However, from an epidemiological point of view, there are crucial questions that need to be answered to determine if these animals are capable of sustaining and spreading *L. infantum* infection (Box 1). Therefore, it is urgent to perform adequate feline and vector studies to clarify the role of cats in the epidemiology of ZL.

Conflict of interest statement

All authors declare that they have no conflicts of interest concerning the work reported in this paper.

Acknowledgments

C.M. (SFRH/BPD/44082/2008) holds a fellowship from Fundação para a Ciência e Tecnologia, Ministério da Ciência, Tecnologia e Ensino Superior, Portugal.

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