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CDM Creador de Motius S.L., Mare de Deu de Montserrat 53-59,
08930 Sant Adrià de Besòs, Barcelona, Spain

ONE HEALTH APPROACH IN LEISHMANIASIS: RESERVOIR ROLE OF *RATTUS NORVEGICUS* IN CITIES

[1]MARÍA TERESA GALÁN-PUCHADES, [2]MERCEDES GÓMEZ-SAMBLÁS, [2]ANTONIO OSUNA, [1,3]JOAN SANXIS-FURIÓ, [4]JORDI PASCUAL, [3]RUBÉN BUENO-MARÍ, [4]SANDRA FRANCO, [4]VÍCTOR PERACHO, [4,5]TOMÁS MONTALVO, AND [1]MÀRIUS V. FUENTES

[1] Grupo de Investigación Parásitos y Salud, Departamento de Farmacia, Tec. Farmacéutica y Parasitología, Facultad de Farmacia, Universitat de València, Burjassot-Valencia; [2] Instituto de Biotecnología, Universidad de Granada, Granada; [3] Laboratorios Lokímica, Catarroja-Valencia; [4] Agència de Salut Pública de Barcelona; [5] CIBER Epidemiología y Salud Pública, Barcelona

Abstract According to the One Health concept, human and animal health are interdependent and connected with the environment in which they coexist. In this context, leishmaniasis is a zoonotic disease that affects humans as well as various wild and domestic mammals. The persistence of the disease in cities, in spite of the control measures implemented in dogs and vectors (phlebotomine sand flies), suggests the existence of a new reservoir. Therein, the presence of *Leishmania infantum* was investigated and quantified by qPCR in the spleens of 98 sewer rats, *Rattus norvegicus* (84 captured in the sewage system and 14 in parks), in the city of Barcelona, Spain. Only 1 rat (7.1%) captured in the parks tested positive for *L. infantum*. However, rats captured in the sewage system showed a 33.3% prevalence (28/84) of *L. infantum* infection and harbored up to 2,272 estimated parasites. According to the obtained results, the absence of surveillance or preventive measures of rat leishmaniasis in urban areas could contribute to the emergence of human and dog infections in cities.

Key words Synanthropic hosts, *Leishmania infantum*, Norway rats, rat leishmaniasis

INTRODUCTION

This research is part of the One Health approach. The One Health concept, introduced by USAID (United States Agency for International Development), WHO (World Health Organization), FAO (Food and Agriculture Organization) and OIE (World Organization for Animal Health), recognizes that human health is connected to the health of animals and the environment in which they coexist. It is estimated that more than 6 of every 10 known infectious diseases in humans are spread from animals, and 3 of every 4 new or emerging infectious diseases in humans are spread from animals. These diseases which spread between humans and animals are known as zoonoses.

Recently, WHO and PAOH (Pan American Health Organization) organized, in Lima (Peru), the first scientific meeting on the problem of rodents and the influence these animals exercise on human health and how to develop a more efficient protection against them, clearly applying the One Health approach. WHO experts estimate that rodents are connected to 40% of zoonoses with about 400 million cases annually. In this context, leishmaniasis is a zoonotic disease caused by infection with *Leishmania* parasites which are spread by the bite of phlebotomine sand flies affecting humans as well as wild and domestic mammals. There are several different forms of leishmaniasis in humans. The most common forms are cutaneous leishmaniasis, which causes skin sores, and visceral leishmaniasis, which affects several internal organs (usually the spleen, liver, and bone marrow).

Leishmaniasis is hypoendemic in Spain (https://www.who.int/leishmaniasis/burden/Leishmaniasis_Spain/en/), a country located in the transmission foci of the Mediterranean basin in which the disease is caused by *Leishmania infantum* that presents a zoonotic life cycle, with dogs being the main known reservoir host. Although considered a rural disease, leishmaniasis is becoming more prevalent in urban areas (Cortes et al, 2007). In cities, dogs are considered the only reservoir of *L. infantum.* Despite the measures taken in big cities (elimination of seropositive dogs, household vector control), leishmaniasis has continued to spread in humans and, in turn, affects new areas (Oliveira et

al., 2008). Concerning the epidemiology of leishmaniasis, the existence of new mammal reservoirs, in addition to dogs, has been highlighted (Miró and López-Vélez, 2018). There are practically no studies regarding the possible reservoir role of synanthropic animals in cities, where only the role of certain domestic mammals, with limited populations such as cats and horses, has been analyzed (Miró and López-Vélez, 2018).

The aim of this study was to investigate and quantify the presence of *Leishmania infantum* in an urban population of *Rattus norvegicus*, the sewer or Norway rat, from Barcelona, employing a highly sensitive real time PCR (qPCR) method for *Leishmania* DNA detection.

MATERIALS AND METHODS

The spleens of a total of 98 *R. norvegicus* were examined to determine the presence of *L. infantum*. The studied areas in Barcelona comprised the sewage system (n=84) as well as parks located in populated residential areas of the city (n=14). In the winter of 2016/17, snap traps were used in the sewage system, wire rat cages were located in parks. Permission for trapping and dissecting was granted by Department of Territory and Sustainability of the regional government of Catalonia (SF/044), according to Directive 2010/63/EU of the European Parliament and Council decision of 22nd September 2010 on the protection of animals used for scientific purposes. The spleens were removed and preserved in 70% ethanol until DNA isolation. DNA was obtained from 10 mg of spleen using the Purification of Total DNA kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA quality and quantity were determined spectrophotometrically in a Nanodrop (Nanodrop ND-1000, Thermo Scientific).

Samples whose DNA concentration was too low with the extraction kit were processed by phenol-chloroform-isoamyl (25:24:1) DNA extraction technique. Quantification of the parasite DNA was by real time qPCR using taqman probe with Fam fluorochrome. Amplification was performed in a CFX96 thermal cycler (Bio-Rad). A 10 μL final volume reaction containing 5 μL SsoAdvanced™ Universal Probes Supermix (Bio-Rad, 1725280), 0.7 μM each primers (Leish1 and Leish2), 0.15 μM of taqman probe (Leishprobe) and 2 μL of spleen DNA (100-200 ng) was prepared. Real time qPCR conditions were 95ºC for 10 min, followed by 40 cycles of 95ºC for 15 sec and 60ºC for 1 min. After amplification, samples were kept at 12ºC. The primers Leish1 (5'-AACTTTTCTGGTCCTCCGGGTAG-3'), Leish2 (5'-ACCCCCAGTTTCCCGCC-3') and Leishprobe (FAM-5'-AAAAATGGGTGCAGAAAT-3'-MGB) were used (Francino et al., 2006). To calculate the number of parasites in the sample, a calibration curve was performed with *L. infantum* cultured in RMPI 1640 medium. An *L. infantum* DNA standard series (10^9 to 10^{-1} equivalent parasites) was included in each calculation for the standard curve, which was calculated by CFX Manager™ Software (Bio-Rad); the minimum amount of DNA amplified equaled 1 parasite (Jara et al., 2013).

RESULTS AND DISCUSSION

One rat captured in the parks was *L. infantum* positive (7.1%). However, rats captured in the sewage system showed a 33.3% prevalence of *L. infantum* infection (28 out of 84). The estimated number of parasites in the positive samples varied considerably, ranging from 0.28 to more than 2,200 (Galán-Puchades et al., 2019). The low number of infected individuals found so far in urban areas has led to the Norway rat being categorized an incidental host, capable of becoming infected but considered irrelevant to the long-term persistence of the disease. Our study demonstrates the importance of the trapping site for finding a large *Leishmania*-infected rat population. No study has analyzed an underground *R. norvegicus* population in sewage systems until now. The sewer rat actively selects this habitat in cities since it provides harborage, food and water, thus being an ideal habitat for rats to either nest or to be used as a network to move around and enter different structures. In addition, a sewage system enables rodents to move above as well as underground and vice versa.

The mere presence of infection in the Norway rat, even in these large qualitative and quantitative numbers, does not necessarily indicate that it is a reservoir host. According to WHO (2010), the incrimination of a particular mammal as *Leishmania* reservoir must depend on an accumulation of evidence. First, the reservoir has to be sufficiently abundant and long-lived. In this sense, *R. norvegicus* is the most abundant mammal, after humans, in cities with a lifespan of around 1-3 years. The lack of predators or interspecific competition in cities guarantees a longer lifetime than in nature. Second, intense host-sand fly contact is necessary. Sewers are breeding sites for *Phlebotomus* species, in which the sand flies reach significant populations (Lucientes et al., 2005). Third, the prevalence of *Leishmania* infection should be more than 20%. Our study revealed a 33.3% prevalence in the sewers. Fourth, the course of infection should be long enough and non-pathogenic to allow the parasites to survive any non-transmission season. Although further studies ought to assess the true pathogenic degree of rat leishmaniasis, we did not find clear

signs of neither hepatomegaly nor splenomegaly in the infected rats. Finally, parasites should be available in the skin or the blood in sufficient numbers to be picked up by a sand fly. However, the availability of the parasite to the sand fly cannot be proved by this study. In naturally infected *R. norvegicus* in Spain and Brazil, *L. infantum* was detected in the hair and blood of the rats, respectively, by molecular methods (Muñóz-Madrid et al., 2013; Lara-Silva et al., 2014).

Only considering the rats from the sewage system, and not those living aboveground, a 0.16 rat-per-person scenario is suspected for Barcelona (Pascual et al., 2019). Therefore, considering this ratio, the prevalence found in the sewers in this study (33.3%) means that there could be more than 86,000 underground leishmaniotic rats in Barcelona (1,620,343 inhabitants in 2018), a figure of public-health concern for a potential reservoir. Our recent unpublished results have demonstrated by molecular methods the presence of *L. infantum*-infected *Phebotomus* in the sewers where the protozoan was also detected in rats. This relevant finding reinforces the fact that *R. norvegicus* should be considered, in addition to dogs, in the control strategies of the disease in urban environments.

CONCLUSION

In conclusion, the, hitherto, absence of surveillance or preventive measures of rat leishmaniasis in urban areas could contribute to the emergence of human and dog infections in cities.

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