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Chagas disease: Importance of rats as reservoir hosts of *Trypanosoma cruzi* (Chagas, 1909) in western Mexico

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ABSTRACT

In Mexico, the role of most species of mammals involved in the transmission cycle of *Trypanosoma cruzi* Chagas, 1909 is poorly known. It was carried out a study to investigate the importance of rats as reservoir of *T. cruzi* in western Mexico, an area with important risk of transmission of *T. cruzi* to human. Thirty-eight human dwellings were searched on two representative towns of western Mexico along twelve months for collection of rats and triatomines. Study rats (*Rattus norvegicus*) Berkenhout, 1769 and triatomines (*Meccus phyllosomus longipennis*) (Usinger, 1939) were collected inside and outside human dwellings. Most rats (68.6%, n = 312) and triatomines (68.7%, n = 217) were collected along months of the hot season. Most rats (59.3%) were collected in peridomiciliary areas. From 312 examined rats, 71 (22.7%) were positive for *T. cruzi* on examination by Indirect Hemagglutination, which was confirmed by xenodiagnosis. From the 217 examined triatomines, 169 (77.9%) were infected by *T. cruzi*. The presence of infected rats and triatomines was highly related since on every studied human dwelling where infected triatomines were collected, infected rats were also found. Rats seem to constitute an important domiciliary and peridomiciliary reservoir for *T. cruzi*, furthering the risk of infection for human beings.

Introduction

In Mexico, Chagas disease, caused by *Trypanosoma cruzi* Chagas, 1909, is considered one of the more important vector-borne diseases. It is estimated that more than five and a half million Mexicans are infected with *T. cruzi* and that there are 69,000 new infections annually. There are a total of 18 endemic areas, including states in the south and southeast, east, center, and western Mexico [1]. In that country, different species of animals have been recorded as serologically positive for infection by *T. cruzi*. Dogs (*Canis familiaris*) Linnaeus, 1758 opossums (*Didelphis* spp.) and rats (*Rattus* spp.) are considered as some of the most important reservoir hosts [1–4]. Dogs, the main domestic reservoir host, represent a risk to human populations due to the high dog/people ratio in some areas (1.7:1–4.6:1), the high fertility of dogs and the close contact between humans and dogs despite the free-roaming nature of the latter [2]. On the other hand, rats, which are considered as one of the most important synanthropic reservoir hosts [5], are also epidemiologically important due to high rat/people ratio in some areas (3:1–5.1:1), the high fertility of rats, and the close contact between rats and humans [6]. In the state of Jalisco, placed in western Mexico, previous studies have shown that rats have higher *T. cruzi* infection rates (35.2–42.9%) than opossums (22–32.4%) and dogs (8.1–10.17%) [7,8]. Complimentary, high infection rate by *T. cruzi* in *Meccus phyllosomus longipennis* (Usinger, 1939), the main

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domiciliary and peridomiciliary triatomine subspecies involved in the transmission of *T. cruzi* in western Mexico [9,10], have been recorded also in the state of Jalisco [9] and it has been recorded that 16–40% of *M. p. longipennis* collected in different areas of that state had fed on rats [11,12], which increases the importance of studying those rodents as reservoir hosts of *T. cruzi*. Besides that, seroprevalence in the human population of Jalisco has reached 12.1%, with several cases of acute human chagasic cardiomiopathy recorded [1]. As a part of a series of studies on the epidemiology of Chagas disease in western Mexico, the occurrence of *T. cruzi* infection in rats and triatomines was investigated.

Material and methods

Study area

Surveys of triatomines and rats infected by *T. cruzi* were conducted within and around two close localities previously reported as with presence of infected triatomines [9] and currently as with a rat infestation problem. Studied localities were Lázaro Cárdenas (20°27′N, 103°58′) and Ipazoltic (20°27′N, 104°00′), both neighboring localities in the municipality of San Martín de Hidalgo, placed in the state of Jalisco, in western Mexico. At the beginning of this study, both villages were mapped, sample size was determined and then, 38 (n = 148) human dwellings were randomly selected by an aleatory number assignment and searched for rats and triatomines for twelve months along 2015. Firstly, each human dwelling was divided into two areas, intradomiciliary and peridomiciliary. The intradomiciliary area accounted for the interior of houses and attached buildings, including rooms circumscribed by the main walls of the dwelling where inhabitants normally sleep. The peridomiciliary area was defined as the area surrounding the homestead. This was usually a fence compound which often included rocks and mounds of construction materials, animal shelters, and agricultural products. Sites beyond fences were considered sylvatic habitats [13].

Biological material (rats)

A rodent survey was carried out by using Live Tomahawk (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) and modified Sherman traps. Twenty traps were placed in the intradomiciliary area and another twenty in the peridomiciliary areas of each searched human dwelling on two consecutive nights on each month. Traps were baited with a piece of bread covered with canned tuna fish conserved in oil, and then placed where rats had been previously observed by inhabitants of the towns under study. Traps were placed at sunset and collected at sunrise to coincide with the rats' nocturnal feeding habits [6]. Collected animals were identified following descriptions specified by Yigit et al. [14] and transported to the laboratory, where a direct blood examination was performed on each one. Fresh tail blood from the captured animals was examined at 400× and in Giemsa-stained smears at 400 and 1000× [15]. Blood flagellates, when found, were identified. Each animal was diagnosed by serological tests, including Indirect Hemagglutination (IHA) (Wiener lab, Buenos Aires, Argentina), and xenodiagnosed by allowing five fourth-instar, laboratory-bred *M. p. longipennis* to engorge upon its blood. Feces of these bugs, spontaneously expelled or obtained by direct abdominal compression were examined and diluted 1:1 in 0.85% saline for up to 30, 45 and 60 days after engorgement. Flagellates were stained with Giemsa in order to study their morphology. A specimen was considered positive if at least two of the three methods returned positive results.

Rats were handled and euthanized following Norma Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio (Technical guidelines for production, care and use of laboratory animals) regulations [16]. Observance of NOM-062-ZOO-1999 was fulfilled by the head of the Committee of Ethical Behaviour of the Centro Universitario del Sur.

Triatomines

Entomological survey was carried out as described by Martínez-Ibarra et al. [13]. Briefly, a three-person team searched all natural and man-made ecotopes of triatomines in human dwellings through 20 min intradomicile and 20 min peridomicile timed manual collection. In addition, 30 wire-netting bait-traps, based on a modified version [13] of Noireau traps [17], were employed. Traps were placed at different sites, including the triatomines natural ecotopes.

All collected triatomines were identified following the keys of Lent and Wygodzinsky [18], considering the revalidation of the genus *Meccus* [19,20] and transported to the laboratory, where they were fed on hens (*Gallus gallus*) (Linnaeus, 1758). Feces of these bugs, spontaneously expelled or obtained by direct abdominal compression were examined and diluted 1:1 in 0.85% saline for up to 30, 45 and 60 days after engorgement. Flagellates were stained with Giemsa in order to study their morphology. Parasites detected in the feces were collected and intraperitoneally inoculated in Swiss mice, which were later euthanized and their organs tested for nests of amastigotes, in order to corroborate that those detected trypanosomatides were *T. cruzi*.

Statistical analysis

The chi-square test was used to compare frequencies. The Pearson correlation coefficient was used to correlated presence of infected rats and triatomines on human dwellings. The Sigma Stat 3.1 software (version 3.1 for windows; Systat Software Inc., San Jose, CA) was used for statistical analysis. Statistical significance level was set at alpha = 0.5.

Results

All collected rats were classified as *Rattus norvegicus* Berkenhout, 1769. Most rats (68.6%, n = 312) and triatomines (68.7%, n = 217) were collected along the hot season (spring and summer times), with significant ($p < 0.05$) differences from cool-cold season (autumn and winter times). Similarly, significantly ($p < 0.05$) more rats (59.9%, n = 312) were collected in peridomiciliary areas than in domiciliary ones. In contrast, slightly more triatomines (52.5%, n = 217) were collected in domestic areas than in peridomestic ones, with not significant ($p > 0.05$) differences between them (Table 1). Thirty-one (24.4%, n = 127) of the collected rats in the domestic area as well as 40 (21.6%, n = 185) rats from the peridomestic areas tested positive by IHA. From those 71 positive rats, the diagnosis was confirmed by xenodiagnosis on 69 of them and by examination of stained blood smears in the other two (Table 2). The flagellates were visible in fresh blood and had the typical morphism of *T. cruzi*. Only one triatomine species was collected, *M. p. longipennis*; 87 (76.3%, n = 114) of examined triatomine specimens from domestic areas and 82 (79.6%, n = 103) from peridomestic areas were infected by *T. cruzi* (Table 1). Only TcI lineage was recorded. All inoculated Swiss mice with those parasites detected in the feces of triatomines were positive for nests of amastigotes, which corroborated that those detected trypanosomatides were *T. cruzi*.

All the studied human dwellings were infested by rats, with a least one specimen positive to *T. cruzi*. In contrast, in only 27 (71.1%, n = 38) of those dwellings were also collected triatomines, with a least one specimen positive to *T. cruzi*. In not a single human

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