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A survey of zoonotic pathogens carried by house mouse and black rat populations in Yucatan, Mexico

J. A. PANTI-MAY[1]*, R. R. C. DE ANDRADE[2], Y. GURUBEL-GONZÁLEZ[3], E. PALOMO-ARJONA[3], L. SODÁ-TAMAYO[3], J. MEZA-SULÚ[3], M. RAMÍREZ-SIERRA[4], E. DUMONTEIL[4,5], V. M. VIDAL-MARTÍNEZ[6], C. MACHAÍN-WILLIAMS[7], D. DE OLIVEIRA[2], M. G. REIS[2], M. A. TORRES-CASTRO[8], M. R. ROBLES[9], S. F. HERNÁNDEZ-BETANCOURT[3] AND F. COSTA[2,10]

[1] *Doctorado en Ciencias Agropecuarias, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Merida, Mexico*
[2] *Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Ministério da Saúde, Salvador, Brasil*
[3] *Departamento de Zoología, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Merida, Mexico*
[4] *Laboratorio de Parasitología, Centro de Investigaciones Regionales 'Dr. Hideyo Noguchi', Universidad Autónoma de Yucatán, Merida, Mexico*
[5] *Department of Tropical Medicine, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA, USA*
[6] *Laboratorio de Patología Acuática, Departamento de Recursos del Mar, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Unidad Merida, Merida, Mexico*
[7] *Laboratorio de Arbovirología, Centro de Investigaciones Regionales 'Dr. Hideyo Noguchi', Universidad Autónoma de Yucatán, Merida, Mexico*
[8] *Laboratorio de Enfermedades Emergentes y Re-emergentes, Centro de Investigaciones Regionales 'Dr. Hideyo Noguchi', Universidad Autónoma de Yucatán, Merida, Mexico*
[9] *Centro de Estudios Parasitológicos y de Vectores, CONICET-Universidad Nacional de La Plata, La Plata, Argentina*
[10] *Instituto de Saúde Coletiva, Universidade Federal da Bahia, Salvador, Brasil*

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SUMMARY

The house mouse (*Mus musculus*) and the black rat (*Rattus rattus*) are reservoir hosts for zoonotic pathogens, several of which cause neglected tropical diseases (NTDs). Studies of the prevalence of these NTD-causing zoonotic pathogens, in house mice and black rats from tropical residential areas are scarce. Three hundred and two house mice and 161 black rats were trapped in 2013 from two urban neighbourhoods and a rural village in Yucatan, Mexico, and subsequently tested for *Trypanosoma cruzi*, *Hymenolepis diminuta* and *Leptospira interrogans*. Using the polymerase chain reaction we detected *T. cruzi* DNA in the hearts of 4·9% (8/165) and 6·2% (7/113) of house mice and black rats, respectively. We applied the sedimentation technique to detect eggs of *H. diminuta* in 0·5% (1/182) and 14·2% (15/106) of house mice and black rats, respectively. Through the immunofluorescent imprint method, *L. interrogans* was identified in 0·9% (1/106) of rat kidney impressions. Our results suggest that the black rat could be an important reservoir for *T. cruzi* and *H. diminuta* in the studied sites. Further studies examining seasonal and geographical patterns could increase our knowledge on the epidemiology of these pathogens in Mexico and the risk to public health posed by rodents.

* Author for correspondence: J. A. Panti-May, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Merida, Mexico.
(Email: panti.alonso@gmail.com)

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INTRODUCTION

The house mouse (*Mus musculus*) and the black rat (*Rattus rattus*) are two of the most widespread mammals in the world [1]. These species are serious pests in urban and rural environments. They are the cause of extensive economic damage to crops, stored food, farms, industries and households [2]. House mouse and black rat populations also harbour and spread zoonotic pathogens, such as viruses (e.g., Seoul hantavirus), bacteria (e.g., *Leptospira interrogans*), protozoa (e.g., *Toxoplasma gondii*) and helminths (e.g., *Hymenolepis* spp.) [3].

Neglected tropical diseases (NTDs) are communicable infections that affect mainly people living in poverty and without adequate sanitation in tropical and subtropical regions [4]. Among these, American trypanosomiasis and leptospirosis are two NTDs that affect millions of people in Latin America [5, 6]. Hymenolepiasis is the most common cestodiasis in humans, particularly children living in areas of low socioeconomic status and low levels of hygiene practices [7, 8]. Although hymenolepiasis is not a NTD, some authors suggest to re-evaluate its status in view of emerging issues relating to the epidemiology and impact on public health of the infection it causes [9].

American trypanosomiasis (Chagas disease), is a zoonotic disease in the Americas caused by the protozoan parasite *Trypanosoma cruzi* [10]. It is endemic in Latin America and continues to be a social and economic problem in many countries, affecting an estimated 6 million people [11]. This disease has two phases, acute and chronic. The acute phase is usually asymptomatic, but when symptoms occur the infection is characterized by an elevated parasitaemia associated with fever, headache, nausea, that is rarely lethal [6]. This phase is followed by a chronic phase, which remains asymptomatic in the majority of patients for life. Approximately 20–40% of patients in this phase present a progressive and debilitating chronic chagasic cardiomyopathy that leads to congestive cardiac failure and death [6]. The transmission to humans is mainly by hematophagous bugs of the genera *Triatoma*, *Panstrongylus* and *Rhodnius* (Hemiptera: Reduvidae). *Trypanosoma cruzi* has been documented in more than 150 domestic animals (e.g. dogs and cats) and wild mammals (e.g. marsupials and rodents). In urban settings, domiciliated and intrusive vectors and synanthropic mammals are involved in the domestic cycle, whereas in rural settings, the cycle is more complex due to the presence of vectors and synanthropic and wild mammals that invade households from (tropical) forests [12]. The black rat and the house mouse have been reported in several countries as important carriers of *T. cruzi* in both domestic and peridomestic cycles [13, 14].

Leptospirosis is a widespread zoonotic disease caused by Gram-negative spirochete bacteria of the genus *Leptospira* [15]. It has been estimated that 1·03 million human cases of leptospirosis and 58 900 deaths due to pulmonary haemorrhage syndrome and acute kidney injury occur annually due to leptospirosis worldwide [16]. *Leptospira* strains (serovars) are, although not totally limited, adapted to different mammalian hosts [3]. For instance, Norway and black rats are reservoirs for the Icterohaemorragiae serogroup, whereas the house mouse is the main reservoir for the Ballum serogroup [15]. In rodents, leptospires cause a systemic infection within 7–9 days after infection but they are rapidly cleared from all tissues except the renal tubules, where bacteria persist and are shed to the environment for several months [17]. Exposure with water or soil contaminated with urine of infected rodents is the common source for human infection. Leptospirosis occurs in diverse epidemiological settings, but in low socioeconomic level/status areas with high abundance of rodents, the risk of *Leptospira* transmission is higher [18]. A 2012 study reported that the median number of leptospirosis cases notified annually in the Americas by national ministries of health was 4713·5 [19].

Human hymenolepiasis is a zoonosis caused by the cestodes *Hymenolepis nana* and *H. diminuta* [20]. Infections with adult hymenolepids occur worldwide, particularly in children [9, 21]. Synanthropic rodents are the main reservoirs for these cestodes [3]. In general, cestodes of the genus *Hymenolepis* require arthropod intermediate hosts in their life cycle, except for *H. nana*, which is the only cestode known to be transmitted directly to another definitive host [22]. In rodents, light infections with *Hymenolepis* are usually non-pathogenic, but heavy infections can cause acute catarrhal enteritis or chronic enterocolitis [22].

Humans can be infected with hymenolepidids by accidental ingestion of intermediate hosts (e.g. beetles or fleas) or by directly ingesting the parasite eggs as a result of contamination of food or water [20]. Human hymenolepiasis is often asymptomatic, but can cause chronic diarrhoea, abdominal pain, irritability and itching [23, 24]. In the Americas, human hymenolepiasis has been reported in several countries, such as Canada, the United States, Mexico, Peru and Argentina [21, 24–26].

In the State of Yucatan, Mexico, it has been estimated that more than 61 000 people are infected with *T. cruzi* [6]. In addition, field studies have reported high abundances of vectors in urban and rural areas [27, 28], and rats being a common blood source for vectors [27]. Epidemiologic studies of human leptospirosis have reported seroprevalences of ~14%, with the icterohaemorrhagiae serovar predominant in the icteric cases [29, 30]. In rodents, *L. interrogans* serovar icterohaemorrhagiae has been reported as the predominant serovar [30, 31]. In Yucatecan children, *H. nana* is a common cestode [32, 33], whereas *H. diminuta* has not been reported. The only study that investigated the helminth fauna of synanthropic rodents, did not reported hymenolepids in black rats nor house mice [34]. The role of synanthropic rodents and polyparasitism in these hosts are vital issues in understanding the epidemiology of these diseases. However, in Mexico, few studies have investigated the role of these animals, especially in the tropical region. The aim of this study was to determine whether house mouse and black rat populations carry *Trypanosoma cruzi*, *Hymenolepis* spp. and *Leptospira* spp. in two urban neighbourhoods and a rural village of Yucatan, Mexico.

METHODS

Study sites

This study was carried out in the residential neighbourhoods of San Jose Tecoh (SJT; 20°53′16·0″N, 89°37′19·9″W) and Plan Ayala Sur (PAS; 20°54′54·0″N, 89°37′22·8″W), in the south of the city of Merida, Yucatan, Mexico. A 2007 study found that *T. dimidiata*, the main vector of *T. cruzi*, infested 38% of houses in the south of Merida and its infection rate by *T. cruzi* was 48% [27]. SJT is an urban area of 1·11 km^2 and ~6001 inhabitants, whereas PAS is a suburban area of 1·32 km^2 and has ~3037 inhabitants [35]. The neighbourhoods are situated in a low socioeconomic level/status area of the south of the city of Merida and are characterized by having paved streets, many small businesses, households in poor conditions (with cracks or holes in doors or windows) and vacant lots. In these neighbourhoods it is common to find pets (i.e. dogs and cats), chickens, weeds, shrubs, fruit trees and unserviceable domestic appliances in the yards. Additionally the rural village of Opichen (OPI, 20°33′05·26″N, 89°51′21·76″W) was surveyed as a part of a collaboration between researchers of the Universidad Autonoma de Yucatan. OPI is a rural area of 1·46 km^2 and has ~4761 inhabitants. This village is located in the western part of the Yucatan. The majority of inhabitants live in houses constructed with stones, wooden poles and thatched with palm leaves that are adjacent to small bedrooms constructed with blocks of concrete. It is common to find chickens, pigs, cattle, weeds, shrubs, trees and vegetable patch plots in the yards.

Trapping methodology

In the two urban neighbourhoods (SJT and PAS), rodents were trapped intensively during a 6-month period from May to October 2013. Thirty households in each neighbourhood were selected at random from spatial maps and sampled monthly. At each household, six Sherman traps (two sizes were used, $8 \times 9 \times 23$ and $8 \times 9{\cdot}5 \times 30{\cdot}5$ cm^3; HB Sherman Traps Inc., Tallahassee, Florida, USA) were set for three consecutive nights [36]. Traps were baited with a mixture of oatmeal and vanilla essence and were distributed in the house and yard close to signs of rodent activity or potential sources of food and/or harbourage. In the rural village (OPI), rodents were non-intensively (one night of trapping) trapped in 50 households in August and September 2013. The rodent trapping was conducted under license from the Mexican Ministry of Environment (SGPA/DGVS/02528/13). Trapped rodents were transported to the laboratory, anaesthetized with an intraperitoneal injection of sodium pentobarbital, and euthanized by cervical dislocation (mice) or with an overdose of anaesthesia (rats) [37].

Data collection

After anaesthesia, a blood sample was obtained by cardiac puncture. Subsequently, animals were euthanized, and heart, kidneys and intestinal tract were removed for pathogen determinations as described below. The blood, heart and kidneys were stored at –80 °C and the

intestinal tract at –20 °C until final use. For financial reasons, not all animals were tested. So, animals were selected at random. Additionally, it was not possible to obtain enough blood and feces samples from all small mammals, especially individuals of the house mouse.

Pathogen survey

Trypanosoma cruzi

The presence of *T. cruzi* DNA in blood and heart samples was detected by polymerase chain reaction (PCR) at the Centro de Investigaciones Regionales 'Dr. Hideyo Noguchi', Mexico. For DNA extraction, we used standardized homemade protocols. Briefly, a half of each heart sample was macerated and homogenized in 400 µl of extraction buffer (1 M Tris–HCl, 5 M NaCl, 0·5 M EDTA, 10% SDS and distilled water). This mixture was allowed to stand at room temperature for 2 h and centrifuged for 10 min at 14 000 rpm. After that, it was transferred to a 1·5 ml microcentrifuge tube with 300 µl of isopropanol and centrifuged at 14 000 rpm. The sediment was dried and re-suspended in 60 µl of TE buffer (0·5 M EDTA 1 M Tris–HCl pH 7·0). To extract DNA from blood, 100 µl of each sample were denaturalized at 95 °C for 10 min in a boiling water bath and centrifuged at 14 000 rpm for 10 min. The supernatant was processed following the methodology described for the heart samples.

In the PCR reaction, we used the primers proposed by Moser *et al.* [38]: TCZ-F and TCZ-R, which amplified a fragment of 188 pb belonging to a region of *T. cruzi* satellite DNA. The reaction (40 µl) included: 1× PCR Buffer (10 mM Tris–HCL pH 8·4 and 50 mM KCl, Promega, USA), 3 mM $MgCl^2$, 0·1 mM dNTP, 250 µM both primers and molecular grade water. The template DNA was used in two different amounts: for heart samples 10 µl were used, whereas for blood samples 1 µl was used. Cycling parameters were one step of 5 min at 94 °C, 35 cycles of 10 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C, and one final extension step of 5 min at 72 °C. All reactions included positive (DNA extracted from a culture of *T. cruzi* lineage I) and negative (sterile water) controls. PCR products were analysed in 1% agarose gels stained with ethidium bromide. Rodents from Opichen were no tested for *T. cruzi*.

Hymenolepis *spp.*

The faecal and caecum contents were examined for *Hymenolepis* eggs using the formalin–ethyl acetate sedimentation technique [39] at the Centro de Investigaciones Regionales 'Dr. Hideyo Noguchi'. One gram of the content was homogenized in a centrifuge tube containing 10 ml of 10% formalin. After homogenization, 3 ml of ethyl acetate were added to the suspension in the tube and the resulting suspension was centrifuged at 1200 rpm for 3 min. Subsequently, the fatty pug was removed and the supernatant discarded. Finally, ∼1 ml of saline solution was added to the sediment and three drops were transferred to a slide for examination. *Hymenolepis* eggs were measured and identified as *H. diminuta* by light microscopy [39].

Leptospira *spp.*

Leptospires in kidneys were detected at the Institute Gonçalo Moniz, Brazil, using the imprint method previously described [40]. Briefly, we obtained kidney imprints by pressure of the cut surface of the tissue onto poly-L-lysine-coated glass slides. Slides were dried at room temperature and fixed in acetone for 3 min prior blocking with 1% bovine serum albumin (BSA) for 40 min. Then they were incubated for 1 h with a primary rabbit polyclonal anti-leptospiral antibody to *Leptospira interrogans* serovar Icterohaemorrhagiae strain RGA diluted 1:1000. Following three phosphate-buffered saline (PBS) washes, the slides were incubated for 1 h with goat anti-rabbit IgG Alexa 488conjugate (Invitrogen, USA) at a 1:500 dilution. After final washings, the slides were mounted with anti-fading medium (ProLong Molecular Probes, Thermo Fisher Scientific, USA) and examined for leptospires using fluorescent microscopy (Olympus BX51 microscope, Olympus America, USA) at a magnification ×400 and ×1000. Samples from non-infected laboratory rats and kidney-positive wild rats were similarly treated as negative and positive controls, respectively. Positive samples were determined by microscopic observation of intact leptospires.

Data analysis

Trap success (TS) was used to estimate the relative rodent abundance as follows: number of rats trapped × 100/(number of traps × number of nights) [41]. The non-parametric Mann–Whitney *U*-test was used to compare the TS between rodent species.

The proportion of positive animals was compared between species and sites, using a Fisher's exact test due to their low frequencies [42]. In all statistical analyses, the level of significance was $P < 0{\cdot}05$.

RESULTS

A total of 302 house mice and 161 black rats were trapped from the three sites (house mice: 159 in SJT, 80 in PAS and 63 in OPI; black rats: 38 in SJT, 109 in PAS and 14 in OPI). The house mouse was significantly more abundant, as suggested by the median trap success, in SJT (TS = 5·2%) than the black rat (TS: 1·1% , $P = 0·005$), whereas in PAS and OPI, the black rat (TS: 3·5% in PAS, 1·2% in OPI) and the house mouse (TS: 2·4% in PAS, 5·3% in OPI) had similar abundances (PAS, $P = 0·093$; OPI, $P = 0·221$). Table 1 shows the number and the percentage of trapped rodents tested for zoonotic pathogens.

Trypanosoma cruzi DNA was detected in 15 of 278 (5·4%) rodent hearts. The overall prevalence in black rats was 6·2% (7/113), whereas in-house mice were 4·9% (8/165) (Table 2). All blood samples tested by PCR were negative. *Hymenolepis diminuta* was the most prevalent pathogen among rodents (5·6%, 16/288). Black rats were more frequently infected with *H. diminuta* (14·2%, 15/106) than house mice (0·5%, 1/182) (Fisher's exact test, $P < 0·001$). Leptospires were detected only in 1 of 118 black rats (0·9%). A co-infection was detected in one individual, a black rat, carrying both *T. cruzi* and *H. diminuta*.

In SJT, 26·9% (7/26) of black rats were positive for *T. cruzi*, whereas in PAS only house mice were found positive (15·7%; 8/51) (Table 2). No animals from OPI were tested for this infection. There was a significant difference in the prevalence of infection with *H. diminuta* in rats and the site of trapping. The prevalence of SJT, 31·1% was higher than the 4·7% of PAS (Fisher's exact test, $P = 0·001$). There were no statistical differences between the prevalence of SJT and OPI ($P = 0·723$), and between OPI and PAS ($P = 0·057$). *Hymenolepis diminuta* eggs were found in a house mouse in OPI (1·2%, 1/52). The sole rat infected with *Leptospira* was trapped in PAS.

Table 1. *Number and percentage (in parenthesis) of house mice and black rats examined for zoonotic pathogens*

	No. of examined rodents		
Pathogen	*Mus musculus* ($n = 302$)	*Rattus rattus* ($n = 161$)	Total
Trypanosoma cruzi			
Blood	233 (77·2)	145 (90·1)	378 (81·6)
Hearts	165 (54·6)	113 (47·8)	278 (60·0)
Hymenolepis spp.	182 (60·3)	106 (65·8)	288 (62·2)
Leptospira spp.	210 (69·5)	118 (73·3)	328 (70·8)

DISCUSSION

The house mouse and the black rat are a threat to public health; however, few studies have evaluated their role as carriers of zoonotic pathogens in urban and rural settlements of Mexico [31, 34, 43, 44]. In this study, we report the presence of *T. cruzi*, *H. diminuta* and *L. interrogans* among house mouse and black rat populations from two urban neighbourhoods and a rural village from Yucatan, Mexico.

In this study, we detected the presence of *T. cruzi* in hearts of house mice and black rats, but not in blood samples. This suggests that rodents were in the chronic phase of the infection, which is characterized by a low parasitaemia and a high invasion of cardiac cells [45, 46]. Several studies have reported that synanthropic rodents are the main reservoir for *T. cruzi* in domestic and peridomestic cycles [47]. Particularly, black rats had a high prevalence (27%), which has been noted in Brazil (24%), Chile (28%), Ecuador (12%) and Yucatan (47%) [13, 14, 48, 49]. Some studies have suggested that the black rat could be a possible link between the domestic and sylvatic cycles of *T. cruzi* due to its synanthropic behaviour, its high reproductive rates and its preference to areas with trees [1]. On the other hand, the house mouse could be an important reservoir in the domestic cycle due to its preference to establish its colonies inside or close to the dwelling and its small home range (3–10 m) [1].

Hymenolepis diminuta was the most prevalent pathogen among rodents, particularly among black rats (14·2%). This parasite, which has a worldwide distribution, parasitizes mainly synanthropic rats of the genus *Rattus* [50]. This cestode has been reported in black rats from different habitats such as households [50], markets [51] and farms [52], with a prevalence varying from 14·3% to 33·3%. In this study, the prevalence among black rat populations varied from 4·7% (95% confidence interval (CI) 1–13·1%) in PAS to 23·1% (95% CI 5·0–53·8%) in OPI and 31·1% (95% CI 15·3–50·8%) in SJT. *Hymenolepis diminuta* requires an arthropod intermediate host to complete its life cycle. The main intermediate hosts are the mealworm beetle (*Tenebrio molitor*), the four beetle (*Tribolium confusum*) and the northern rat flea (*Nosopsyllus fasciatus*) [22]. The variation found in the prevalence could be related to the abundance of intermediated

Table 2. *Prevalence of zoonotic pathogens in house mice and black rats from Yucatan, Mexico*

	San Jose Tecoh		Plan de Ayala Sur		Opichen		Total	
Pathogen	House mice	Black rats	House mice	Black rats	House mice	Black rats	House mice	Black rats
Trypanosoma cruzi	0 (0/114)	26·9 (7/26)	15·7 (8/51)	0 (0/87)	–	–	4·9 (8/165)	6·2 (7/113)
Hymenolepis diminuta	0 (0/70)	31·1 (9/29)	0 (0/60)	4·7 (3/64)	1·2 (1/52)	23·1 (3/13)	0·5 (1/182)	14·2 (15/106)
Leptospira interrogans	0 (0/93)	0 (0/32)	0 (0/62)	1·4 (1/73)	0 (0/55)	0 (0/13)	0 (0/210)	0·9 (1/118)

Data are presented as % positive (*n* positive/*N* analysed).

hosts in each area. Further studies investigating the species and abundance of intermediate hosts present in the studied sites could help us to understanding the epidemiology of *H. diminuta* in Yucatan.

The leptospiral carriage among *R. rattus* trapped in this work was low (0·9%). In a rural community close to the sampled neighbourhoods (San Jose Tecoh and Plan de Ayala Sur), *L. interrogans* was reported with a prevalence of 12·8% in *R. rattus* by PCR [31]. Although *R. rattus* has been reported as carrier of pathogenic *Leptospira*, in the Americas several studies have reported that *R. norvegicus* is the main reservoir in urban slums of Brazil, Colombia and Peru [53–55]. The low prevalence of *Leptospira* in *R. rattus* could be explained by the fact that *R. rattus* is an arboreal animal in contrast to *R. norvegicus* that is typically more terrestrial [1]. In Yucatan, there are no records of *R. norvegicus*, which suggests that *R. rattus* could be the main reservoir of *Leptospira* in absence of *R. norvegicus* as has been noted in some islands [56, 57].

In this study we used different methodologies to detect different pathogens. PCR amplification of the 188 pb *T. cruzi* repetitive element is a highly sensitive technique for detecting small numbers of parasites, only a 1/200 of the DNA of the parasite is necessary for a positive identification [38]. However, it is more applicable for acute infections than in chronically infected mammals; in chronically mammals, parasitaemias are intermittent and contain few or no parasites [38]. On the other hand, *T. cruzi* lineage I, the predominant lineage in Mexico, has a tropism for the cardiac cells during the chronic phase of the infection [58], which indicates the utility of PCR for detection of tissue parasite in chronically infected hosts [47]. The formalin–ether/ethyl acetate is a widely used sedimentation technique for the diagnosis of intestinal parasite eggs [39, 59]. Its sensitivity ranging from 72% to 85%, depending on several factors such as the parasite species, the number of eggs/cysts per gram of faeces, and the time of infection [59]. For *H. nana*, this technique has shown a sensitivity ranging from 61% to 72% [59, 60]. The immunofluorescent imprint method is a rapid technique for the direct observation of *Leptospira* spp. by microscopy. This method has been used to study experimental and natural infections [40, 61]. A comparative study with the real-time PCR (qPCR) showed that for the detection, the imprint method is equivalent to qPCR in both acute and chronic rodent models [62]. Nevertheless, this method was restricted to the serovar Icterohaemorrhagiae, and consequently the prevalence of *Leptospira* could be underestimated. As the capacity to detect parasites is different between techniques, prevalence data of the three parasites are not comparable and may be compared only with studies using similar techniques.

Several studies have reported that changes in rodent demography, intermediate host populations and environmental factors could alter the risk of zoonotic pathogen transmission [61, 63]. In this study, we found an overall low prevalence of zoonotic pathogens in rodent populations; however, previous ecological studies in Merida, Yucatan have shown that the reproductive rates of synanthropic rodents are high in low socioeconomic areas, which could increase the public health risks. Of the pathogens examined, *T. cruzi* and *H. diminuta* could represent a risk to inhabitants. *Trypanosoma cruzi* is a serious threat in Latin America due to the irreversible damage caused by the parasite, the low efficacy of the antiparasitic treatment during the chronic phase of the disease, and the presence of intrusive vectors, which lead to considerable morbidity and mortality rates [6]. Case reports of *H. diminuta* infection in humans are uncommon and are limited to rural and urban areas with high levels of poverty; however, in these areas, the environmental characteristics favour the abundance of rodents and intermediate hosts, facilitating the reinfection [24, 64]. Conversely, *L. interrogans* was the less prevalent pathogen among rodents. Further

studies are required to assess whether humans are becoming infected within the studied sites. Our results suggest that the black rat could be an important reservoir for *T. cruzi* and *H. diminuta* in the studied sites. Nevertheless, both mice and rats live in close contact with inhabitants invading kitchens, bedrooms and consuming human foodstuff, which could increase the risk for a pathogen to be transmitted to inhabitants. It would be advisable to conduct further studies examining seasonal and geographical patterns. This could increase our knowledge on the epidemiology of these pathogens in Mexico.

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DECLARATION OF INTEREST

None.

ETHICAL STANDARDS

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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