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Molecular identification of *Blastocystis* sp. in urban rodents from México City

Identificación molecular de *Blastocystis* sp. en roedores urbanos de la Ciudad de México

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Urban rodents are synanthropic animals that play a pivotal role as hosts for rodent-transmitted diseases; however, there are few reports of intestinal zoonotic protozoa in these animals. On the other hand, *Blastocystis* sp. is the most frequently identified protozoan zoonotic parasite in coprological studies of humans worldwide, and its presence in urban rodents has only been documented in some studies. The present study aimed to identify *Blastocystis* sp. by molecular tools in a population of synanthropic rodents from México City. Eighty-five rodents (33 *Mus musculus* and 52 *R. norvegicus*) were trapped in the Canal Nacional public park in México City, during the fall and winter of 2020. Some morphological data and large intestine samples were obtained with feces from all the animals. DNA was recovered from the samples and processed using the polymerase chain reaction technique for the identification of *Blastocystis* sp. Twenty-one samples were positive, so the prevalence was 32.7 % and 12.1 % for *R. norvegicus* and *M. musculus*, respectively. Since a previous report has documented a frequency of 31.5 % of *Blastocystis* sp. in patients from México City, similar to the frequency found in rats in the present study and in agreement with other authors, our results strengthen that *R. norvegicus* could be used as a sentinel synanthropic animal; however, studies on human and rodent ecology should be carried out to confirm this.

Key words: *Blastocystis* sp.; *Mus musculus***;** protozoan; *Rattus norvegicus*; synanthropic; zoonoses.

Los roedores urbanos son animales sinantrópicos que desempeñan un papel fundamental como hospederos de las enfermedades transmitidas por roedores; sin embargo, existen escasos informes de protozoos zoonóticos intestinales en estos animales. Por otro lado, *Blastocystis* sp. es el parásito zoonótico protozoario identificado con mayor frecuencia en estudios coprológicos de humanos a nivel mundial, y su presencia en roedores urbanos solo se ha documentado en algunos estudios. El objetivo del presente estudio fue identificar a *Blastocystis* sp. mediante métodos moleculares en roedores sinantrópicos de la Ciudad de México. Ochenta y cinco roedores (33 *Mus musculus* y 52 *Rattus norvegicus*) fueron atrapados en el parque público de Canal Nacional en la Ciudad de México, durante el otoño e invierno de 2020. Se obtuvieron muestras de intestino grueso de todos los animales. Se recuperó DNA de las muestras y se procesaron mediante la técnica de reacción en cadena de la polimerasa para la identificación de *Blastocystis* sp. Veintiún muestras fueron positivas, por lo que la prevalencia fue del 32.7 % y 12.1 % para *R. norvegicus* y *M. musculus*, respectivamente. Un reporte previo documentó una frecuencia del 31.5 % de *Blastocystis* sp. en pacientes de la Ciudad de México con trastornos gastrointestinales, y en concordancia con otros autores, nuestros resultados fortalecen que *R. norvegicus* podría ser utilizado como animal sinantrópico centinela; sin embargo, estudios que profundicen en la ecología humana y del roedor son necesarios para asegurar esto.

Palabras clave: *Blastocystis* sp; *Mus musculus*; protozoario; *Rattus norvegicus*; sinantrópico; zoonosis.

Blastocystis sp. is a protozoan parasite that inhabits the intestines of a wide range of animals, including vertebrates and invertebrates; it has a worldwide distribution with high prevalence, mainly in developing countries (Tan 2008). This parasite is transmitted by fecal-oral route and cross-transmission among hosts is possible (Tan 2008). Pathogenesis of this parasite in humans is still under debate (Olyaiee *et al.* 2022), even when some gastrointestinal effects have been related to it and some experimental pathology has been demonstrated in laboratory models (Ajjampur and Tan 2016). In addition, *Blastocystis* sp. exhibits a high morphological and genetic polymorphism, *i.e.*, it has 4 infection stages today recognized: vacuolar, also named "central body"; granular; amoeboid and cyst (Tan 2008); the vacu-

olar form is the most common stage observed in feces of infected hosts, this size can range from 10 to 200 µm, causing mistakes in its identification during coprological diagnosis, since it is frequently confused with yeasts or cysts of another protozoan (Stensvold *et al.* 2009). *Blastocystis* sp. genetic polymorphism is wide, it has up to 48 ribosomal lineages known as subtypes (STs) that have been described by analyzing the small subunit ribosomal DNA (SSU-rDNA; Maloney *et al.* 2022; Santin *et al.* 2024) and certain STs show moderate specificity towards human and animal hosts; however, cryptic host specificity exists for at least some of them (Martínez-Hernández *et al.* 2020; Stensvold and Clark 2020); although, the role of genetic variability and STs in the pathogenesis, transmission, and epidemiology of *Blastocystis* sp. is still unclear.

The role of synanthropic species as carriers or reservoir hosts of zoonotic pathogens has been well documented; urban rodents, for instance, are a clear example of synanthropic animals that harbor significant zoonotic diseases (rodent-borne diseases, RBD; Hassell *et al.* 2017; Galán-Puchades *et al.* 2021). Two important urban rodents are the house mouse (*Mus musculus*) and the brown rat (*Rattus norvegicus*) originally from Asia and now distributed all around the world. These species are proposed as obligate pests because of their dependency on human settlements; reproduction, behavior, density, and movements of these rodents are strongly associated with human infrastructure and resources (Feng and Himsworth 2014; Vadell *et al.* 2014). *Mus musculus* is a small rodent, less than 250 mm in total length with color from grey to black (Godinez and Guerrero 2014; Islam *et al.* 2021). *Rattus norvergicus* is a big rodent, bigger than 250 mm in total length, it could be confused with *Rattus rattus*; however, they differ in the ears which are smaller (< 20 mm) and the tail is short than the head and body length for *R. norvergicus* (Godinez and Guerrero 2014; Islam *et al.* 2021).

For the particular case of *Blastocystis* sp. in urban rodents, a study performed in a population of *R. norvegicus* from Barcelona, Spain, using molecular techniques, identified to *Blastocystis* sp. in 83.5 % of animals (Galán-Puchades *et al.* 2021). In another study, from 127 fecal samples of urban *R. norvegicus* from Iran, 15.8 % were detected as positive by nested-PCR (Mohammadpour *et al.* 2020) even when this is not full evidence of reservoir capacity, is an indication of contact with the parasite which is a step to determining host capacity. The aim of the present study was to identify *Blastocystis* sp. infection in a population of synanthropic rodents from México City by molecular tools.

The study was done during fall and winter of 2020, as part of an ecological restoration program realized along an open water channel "Canal Nacional" (approximately at 19º 21' 02" N, 99º 07' 11" W; Figure 1); local authorities of México City together with Universidad Autónoma Metropolitana (UAM), perform a control program target pest rodent, carried out under the 31112246 approval project within the agreement UAM-SAREVICH 322003 and under the observance of Guidelines of the American Society of Mammalogists for the use of wild mammals in research (Gannon *et al.* 2007) and the Mexican normative for Lab Animals (Norma Oficial Mexicana NOM-062-ZOO-1999, SADER 2001).

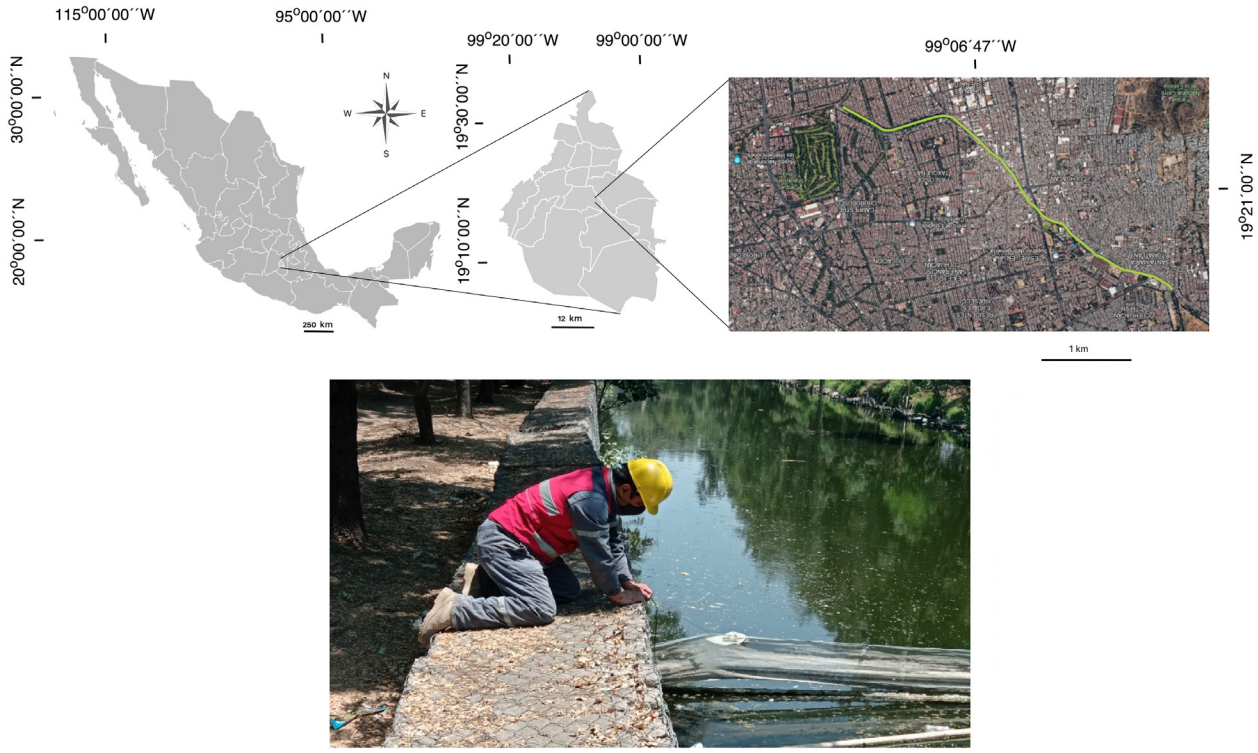

Figure 1. Investigation region, up from left to right: country-México, state-México City, and studied area-open water channel "Canal Nacional". Down: trap placement.

Rodents were captured using commercial box traps of gauge galvanized wire mesh (Tomahawk Live Tramp-like 30 x 20 x 14 cm), baited with oats, vanilla essence, peanut butter and corn tortilla, anesthetized with chloroform and euthanized by cervical dislocation according to SADER (2001). Specimens were transferred to the laboratory for their morphological species identification, age classification (using weight and body length) and sex record (Bjornson *et al.* 1973). The dissections of large intestines were carried out by surgically opening of the bodies and intestine samples were stored frozen at -20 °C until use.

DNA was extracted from approximately 100 mg of each intestine sample; these were incubated with 800 µL of lysis solution (50 mM Tris-HCl, 50 mM EDTA at pH 8, 50 mM NaCl, 1 % SDS, and 20 µg/mL Proteinase K) at 55 °C overnight. The DNA was isolated using a phenol-chloroform technique (Sambrook *et al.* 1989) and then stored at 20 °C until use. DNA concentration was determined by UV spectrophotometry. Molecular identification was performed by amplifying a region of the SSU-rDNA using previously reported primers (Santin *et al.* 2011). The primers used (Blast F 505: 5′- GGA GGT AGT GAC AAT AAA TC -3′ and Blast R 998: 5′- TGC TTT CGC ACT TGT TCA TC -3′) for end-point PCR assays amplify an ~ 500 bp region. PCR amplifications were carried out in a final volume of 25 µL containing 5 pmol of each primer, 1× PCR buffer (8 mM Tris-HCl, pH 8, 20 mM KCl), 1.5 mM $MgCl_2$, 0.5 mM dNTPs, and 1.25 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). Up to 200 ng of DNA was used as a template to amplify genomic sequences. PCR cycling conditions were: initial denaturation at 95 °C for 5 min; followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 56 °C for 30 sec and extension at 72 °C for 30 sec ending with a final extension at 72 °C for 7 min. PCR products were analyzed with 1.2 % agarose gel electrophoresis and visualized by ethidium bromide staining (0.5 µg/ mL).

Prevalence and Confidence Interval 95 % Fisher Exact (Clopper-Pearson) were calculated by species and then layered by sex and age. Contrast by species, sex and age was done by Xi^2 test or Fisher Exact test when the number of individuals to contrast was $n < 5$ by category. All analyses were done with the open software OpenEpi® (Dean *et al.* 2013).

Intestinal samples from 33 *M. musculus* and 52 *R. norvegicus* were studied. Most of the rodents were adults (26 *M. musculus* and 38 *R. norvegicus*); in mice, the number of males analyzed was greater than females (12F:21M), in contrast, for rats there were more females than males (30F:22M). From the adult female 6 mice and 3 rats were pregnant or lactation.

Twenty-one amplicons were obtained; thus, overall *Blastocystis* sp. prevalence was 24.7 % (CI 95 % 16.0-35.3, $n = 85$); meanwhile, for *R. norvegicus* was 32.7 % (CI 95 % 20.3-47.1, $n = 52$) and 12.1 % (CI 95% 3.4-28.2, $n = 33$) for *M. musculus,* the difference in prevalence between species was statistically significant ($Xi^2 = 3.553$, $P = 0.029$). Prevalence by sex and age and combination between them showed slight variation in males and adults slightly higher, but not significant difference was found. Details of each group´s prevalence are presented in Table 1.

In México, as in other countries, reports of RBD in *R. norvegicus* from urban areas are scarce and practically absent with intestinal zoonotic protozoans, since the most of these studies have been carried out in rodents from rural or wild environments (García-Prieto *et al.* 2012; Panti-May *et al.* 2021); thus, in concordance, there are few reports regarding to *Blastocystis* in rodents tramped in cities.

We performed a molecular diagnostic to detect *Blastocystis*, this technique has the highest sensitivity compared to microscopy or *in vitro* culture to diagnostic for animals and humans (Roberts *et al.* 2011; Süli *et al.* 2018). The molecular technique should be the gold standard for this parasite because of its sensitivity but also because nowadays is cheap and easy to perform making more uniform results, unlike microscopy despite is cheap but needs highly skilled personnel to be performed since the parasite is morphologically polymorphic, also this cause an observational bias (Süli *et al.* 2018).

In the present study, we found that the *Blastocystis* prevalence was 32.7 % for *R. norvegicus* and 12.1 % for *M. musculus*. A study performed in feces of urban *R. norvegicus* from Kuala Lumpur (Malaysia) found that prevalence of *Blastocystis* sp. was 51 %, diagnosed by microscopy observation (Premaalatha *et al.* 2017). As previously noted, studies carried out in Spain and Iran cities analyzing fecal samples from *R. norvegicus* by molecular techniques for *Blastocystis*

Table 1. Prevalence of *Blastocystis* sp. layered by species, sex, and age. Bold *P* value shows significant difference. NA = not applicable.

	n	Prevalence %	IC 95 %		*P* value
Overall	85	24.7	16.0	35.3	
Rats	52	32.7	20.3	47.1	**0.030**
Mice	33	12.1	3.4	28.2	
		Rats			
Female	30	30.0	14.7	49.4	0.427
Male	22	36.4	17.2	59.3	
Adult	38	34.2	19.6	51.4	0.480
Young	14	28.6	8.4	58.1	
Adult Female	21	38.1	18.1	61.6	0.414
Adult Male	17	29.4	10.3	56.0	
Young Female	9	11.1	0.3	48.3	0.095
Young Male	5	60.0	14.7	94.7	
		Mice			
Female	12	8.3	0.2	38.5	0.480
Male	21	14.3	3.0	36.3	
Adult	26	15.4	4.4	34.9	0.325
Young	7	0.0	0.0	41.0	
Adult Female	11	9.1	0.2	41.3	0.416
Adult Male	15	20.0	4.3	48.1	
Young Female	1	0.0	0.0	97.5	NA
Young Male	6	0.0	0.0	45.9	

identification, reported prevalence of 83.5 % and 15.8 %, respectively (Mohammadpour *et al.* 2020; Galán-Puchades *et al.* 2021). In our knowledge, the frequency of *Blastocystis* in naturally infected urban *M. musculus* has not been reported. However, experimental infection by this microorganism in albino mice has been well documented (Moe *et al.* 1997; Tan 2008; Elwakil and Hewedi 2010).

Interestingly, a study performed in patients with intestinal disorders, who received medical attention in a hospital at south of México City, reported a *Blastocystis* prevalence of 31.1 % (Jiménez-González *et al.* 2012); this data could point to urban *R. norvegicus* as environmental-sentinel animals for certain parasitic zoonosis, such as *Blastocystis* infection. However, to analyze *Blastocystis* transmission from *R. norvegicus* to humans, much more elements are needed. *Blastocystis* is transmitted by fecal-oral route (Tan 2008), it implies that feces must have contact with oral mucosa directly or indirectly (*e.g.,* feces contaminated drinking water or food). The particular situation of the rodents in the "Canal Nacional" makes difficult the transmission from rodents to humans because rats usually have a small home range in urban areas, limited by human infrastructure like streets or in this case the water channel, making dispersion unlikely (Feng and Himsworth 2014). In addition to this, a meta-analysis of drivers for *Blastocystis* spillover, found that geographical overlapping was one of the most important drivers (Wilcox *et al.* 2021); therefore, for transmission to take place, rats should be inhabiting the same area as humans, therefore animals inhabiting areas outdoor represent a minor transmission risk (Feng and Himsworth 2014).

On the other hand, the lower prevalence of this parasite in mice can be explained because the murine model of blastocystosis showed some degree of resistance (Moe *et al.* 1997). Despite the lower prevalence, mice could play an important role in the *Blastocystis* to human transmission because of a higher occurrence of *M. musculus* indoor house than *R. norvergicus* (Langton *et al.* 2001).

Finally, we identify *Blastocystis* sp. infection in synanthropic rodents, which confirms the rodents´ participation in the *Blastocystis* sp. cycle. However, the present study exhibits some potential biases, the small area and sample size studied for both rodent species and the non-identification of *Blastocystis* subtypes. These biases limited our understanding of the exact role of rodents in the maintenance and transmission of *Blastocystis* sp., for this it is advisable to carry out causality studies on *Blastocystis* infection in synanthropic rodents, as well as expand the search for other RBD, following the recommendation of the One Health approach to improve human, animal, and environmental health. This would include the ecology of the rodents in the city, human ecology, as well as the evaluation of the impact of the ecological restoration.

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Literature cited

Ajjampur, S. S., and K. S. Tan. 2016. Pathogenic mechanisms in *Blastocystis* spp.—Interpreting results from *in vitro* and *in vivo* studies. Parasitology International 65:772-779.

Bjornson, B. F., *et al.* 1973. Control of domestic rats & mice. Atlanta: CDC 4-6. https://stacks.cdc.gov/view/cdc/7668.

Dean, A. G., *et al.* 2013. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 2013/04/06. https://www.openepi.com/Menu/OE_Menu.htm. Accessed on January 18, 2024.

Elwakil, H. S., and I. H. Hewedi. 2010. Pathogenic potential of *Blastocystis hominis* in laboratory mice. Parasitology Research 107:685-689.

Feng, A. Y., and C. G. Himsworth. 2014. The secret life of the city rat: a review of the ecology of urban Norway and black rats (*Rattus norvegicus* and *Rattus rattus*). Urban Ecosystems 17:149-162.

Galán-Puchades, M. T., *et al.* 2021. One Health Approach to Zoonotic Parasites: Molecular Detection of Intestinal Protozoans in an Urban Population of Norway Rats, *Rattus norvegicus*, in Barcelona, Spain. Pathogens 10:311.

Gannon, W. L., *et al.* 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809-823.

García-Prieto, L., *et al.* 2012. Helminth parasites of wild Mexican mammals: list of species, hosts and geographical distribution. Zootaxa 3290:1-92.

Godinez, E. G., and S. Guerrero. 2014. Los roedores de Jalisco, México: clave de determinación. Therya 5:633-678.

Hassell, J. M., *et al.* 2017. Urbanization and disease emergence: dynamics at the wildlife–livestock–human interface. Trends in Ecology & Evolution 32:55-67.

Islam, M. M., *et al.* 2021. Morphometric study of *Mus musculus*, *Rattus norvegicus*, and *Rattus rattus* in Qatar. Animals 11:2162.

Jiménez-González, D. E., *et al.* 2012. *Blastocystis* infection is associated with irritable bowel syndrome in a Mexican patient population. Parasitology Research 110:1269-1275.

Langton, S. D., *et al.* 2001. The occurrence of commensal rodents in dwellings as revealed by the 1996 English House Condition Survey. Journal of Applied Ecology 38:699-709.

Maloney, J. G., *et al.* 2022. Identification and molecular characterization of four new *Blastocystis* subtypes designated ST35-ST38. Microorganisms 11:46.

Martínez-Hernández, F., *et al.* 2020. Molecular genotyping of *Blastocystis* spp. in wild mammals from México. Parasitology Research 119:97-104.

Moe, K. T., *et al.* 1997. Experimental *Blastocystis hominis* infection in laboratory mice. Parasitology Research 83:319-325.

Mohammadpour, I., *et al.* 2020. First molecular subtyping and phylogeny of *Blastocystis* sp. isolated from domestic and synanthropic animals (dogs, cats and brown rats) in southern Iran. Parasites and Vectors 13:1-11.

Olyaiee, A., *et al.* 2022. Gut Microbiota Shifting in Irritable Bowel Syndrome: The Mysterious Role of *Blastocystis* sp. Frontiers in Medicine 9:890127.

Panti-May, J. A., *et al.* 2021. A checklist of the helminth parasites of invasive murid rodents in México. Therya 12:85-93.

Premaalatha, B., *et al*. 2017. A survey of endoparasite and ectoparasite infections of wild rats caught of Ipoh and Kuala Lumpur, Malaysia. Malaysian Journal of Veterinary Research 8:29-34.

Roberts, T., *et al*. 2011. Comparison of microscopy, culture, and conventional polymerase chain reaction for detection of *Blastocystis* sp. in clinical stool samples. The American Journal of Tropical Medicine and Hygiene 84:308.

Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. (SADER). 2001. NOM-062-ZOO-1999. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio.

Sambrook, J., *et al*. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor. New York, U.S.A.

Santin, M., *et al*. 2011. Development of a new PCR protocol to detect and subtype *Blastocystis* spp. from humans and animals. Parasitology Research 109:205-212.

Santin, M., *et al*. 2024. Maloney J. G. Division of *Blastocystis* ST10 into three new subtypes: ST42-ST44. Journal of Eukaryotic Microbiology 71:e12998.

Stensvold, C. R., *et al*. 2009. Pursuing the clinical significance of *Blastocystis*-diagnostic limitations. Trends in Parasitology 25:23-29.

Stensvold, C. R., and C. G. Clark. 2020. Pre-empting Pandora's box: *Blastocystis* subtypes revisited. Trends in Parasitology 36:229-232.

Süli, T., *et al*. 2018. Comparison of conventional and molecular diagnostic techniques for detection of *Blastocystis* sp. in pig faeces. Iranian Journal of Parasitology 13:594-601.

Tan, K. S. 2008. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clinical Microbiology Reviews 21:639-665.

Vadell, M. V., *et al*. 2014. Are life-history strategies of Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*) dependent on environmental characteristics? Wildlife Research 41:172-184.

Wilcox, J. J., *et al*. 2021. Historical contingency, geography and anthropogenic patterns of exposure drive the evolution of host switching in the *Blastocystis* species-complex. Parasitology 148:985-993.

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