Hindawi
Journal of Parasitology Research
Volume 2019, Article ID 7409076, 6 pages
https://doi.org/10.1155/2019/7409076

Research Article

Screening of Zoonotic Parasites in Playground Sandboxes of Public Parks from Subtropical Mexico

Gonzalo A. Pacheco-Ortega,[1] **José I. Chan-Pérez**,[1] **Antonio Ortega-Pacheco**,[2] **Eugenia Guzmán-Marín**,[1] **Melissa Edwards**,[3] **Mark A. Brown**,[3] **Matilde Jiménez-Coello**,[1] **and Ivonne B. Hernández-Cortazar**[1]

[1]*Laboratorio de Biología Celular, Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autónoma de Yucatán, 97000 Mérida, Mexico*
[2]*Depto. de Medicina Interna y Cirugía. Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, 97100 Mérida, Mexico*
[3]*Office for Undergraduate Research and Artistry, The Institute for Learning and Teaching, Colorado State University, Fort Collins, CO 80521, USA*

Correspondence should be addressed to Ivonne B. Hernández-Cortazar; ivonne.hernandez@correo.uady.mx

Received 27 February 2019; Accepted 14 April 2019; Published 2 June 2019

Academic Editor: Kwang Poo Chang

The pathological agents *Toxoplasma gondii, Ancylostoma caninum,* and *Toxocara canis* are widely distributed zoonotic parasites with high prevalence in tropical and subtropical regions of the world. The aim of the present study was to determine the presence of DNA from these parasites in sand samples from the sand playgrounds in the southeastern region of Mexico. Samples of sand were collected from 68 playgrounds in public parks in the city of Merida, Yucatan, which is the main urban area in the southeast of Mexico. The samples were examined using nested PCR to detect the SAG1 gene from *Toxoplasma gondii*, and endpoint PCR for the amplification of ITS-2 and rRNA-ITS2 genes from *Toxocara canis* and *Ancylostoma caninum,* respectively. The presence of *T. gondii* DNA was detected in 11.8% (8/68) samples, DNA from *A. caninum* and *T. canis* was not detected. Results indicate that playgrounds from the studied sandboxes are contaminated with *T. gondii* oocysts and may represent a risk of infection for people in contact with the sand, especially for preschoolers.

1. Introduction

Parasitic zoonoses transmitted by cats and dogs represent a serious problem of public health. The overpopulation of stray dogs and cats which exists predominantly in metropolitan areas increase the risk of contamination of public spaces with infected stools. Among the principal zoonotic parasites transmitted by cats, *Toxoplasma gondii* is one of the most commonly reported and *Toxocara canis* and *Ancylostoma caninum* are common in dogs, particularly when free roaming and without the basic preventive medicine [1, 2].

The obligate intracellular protozoan *T. gondii* has a worldwide distribution and is capable to infect humans and all warm-blooded animals including mammals and birds. It is estimated that one-third of the world population have been exposed to *T. gondii* [3], and the highest prevalence have been reported in countries from Latin America and African tropical countries [4]. Felids are the key animal species in the life cycle of *T. gondii* because they are the hosts which allows the completion of sexual reproduction in their gut and later are able to shed the environmentally resistant stage, the oocyst [5], after a primary infection felids are capable of shedding up to 10 million oocysts in just one day and usually only shed the organism for a short period of time [6]. On the other hand, *T. gondii* oocysts are highly resistant to warm and humid conditions, staying viable in soil for up to 21.5 months [7]. This highlights the risk that they represent to acquire the infection. The majority of horizontal

transmissions to humans are caused either by the ingestion of tissue cysts in undercooked infected meat or by the ingestion of water, food, or soil contaminated with sporulated oocysts derived from feline feces [8]. In Europe, it has been reported that the ingestion of sporulated oocysts represents 6-17% of infections in pregnant women [9], constituting an important way of transmission. The presence of *T. gondii* oocysts in environmental samples has been reported in China and France with positive results in soil samples of 12.69 and 29.2%, respectively [10, 11]. A study carried out in Brazil isolated DNA of *T. gondii* in 25.8% of 31 samples obtained from playgrounds of elementary public schools [12]. Numerous outbreaks of symptomatic toxoplasmosis related to the ingestion of oocysts from soil contamination have been reported [13–15].

Toxocara canis is an ascarid nematode with a worldwide distribution; their definitive hosts are the domestic dog and cat. Infection occurs when the definitive host and the intermediate host (humans) ingest the embryonated eggs of the parasite from contaminated sources (e.g., soil); this contamination is the result of the indiscriminate defecation by the definitive hosts. These eggs can remain viable for months to years outside of the host due to a resistant outer shell composed of ascarosides; this layer enables eggs to withstand various harsh chemicals, extreme temperature changes, and various degrees of moisture [16]. It has been recognized that the use of public parks is an important risk factor for the acquisition of *T. canis* infections in children [17]. The prevalence of infection in humans is variable; it tends to be smaller in industrialized countries (0.7-2.4%) when compared with less industrialized ones (63.2-92.8%) [18]. In Mexico, studies have reported a prevalence of 12.02 to 22.2% in children [19, 20]. Also, studies have been conducted for the detection of eggs from the parasite in public parks from Portugal, Poland, Ireland, and Mexico among others, with a prevalence of 85.7%, 53%, 15%, and 60%, respectively [21–24].

Ancylostoma caninum is also a nematode parasite with the dog being its definitive host. Infection occurs when the host eliminates eggs through defecation; 5 to 10 days later, they transform into their infective stage of filariform larvae which can invade humans via hair follicles and trough small fissures on skin until they reach the small bowel, their definitive habitat. In humans, this can cause an eosinophilic gastroenteritis and chronic iron deficiency anemia which can result in long-term poor health outcomes like reduced cognitive, intellectual, and physical development and reduced fertility among women [25, 26]. Environmental contamination has been evaluated in multiple countries and tends to be higher in tropical regions. Argentina, Brazil, and Venezuela reported prevalence rates of 20.5, 64.8, and 61.1%, respectively, while countries like Spain, Italy, and Poland reported much lower prevalence, 3, 7, and 3.2%, respectively [27].

The southern region of Mexico is considered an endemic area for these zoonoses where prevalence of infection in humans have been reported to be 90% for *T. gondii* and 29.2% for *T. canis* [28], and with regard to *A. caninum,* there are no available studies yet. However, studies evaluating environmental contamination with these parasites in this region are scarce. In a study conducted in a region of southern Mexico (Campeche city), the contamination with *A. caninum* of 92.8% of stools collected from 14 public parks was reported [29]. In the study region, the presence of *A. caninum* and *T. canis* eggs was reported in dog stool samples of 73.8 and 6.2%, respectively [30]. Only one study has been made in this region with regard to the environmental contamination with *T. gondii*, finding the presence of DNA in 5.4% of samples of public drinking water in an urban area [31]. No studies evaluating the presence of DNA nematodes in soil samples from public parks have been reported; instead, studies generally report the presence and abundance of eggs. Therefore, the aim of this study was to determine the presence of *T. gondii*, *T. canis,* and *A. caninum* DNA in sand samples of playground sandboxes from public parks in southeastern Mexico.

2. Materials and Methods

2.1. Study Area and Sampling. This study was conducted in the city of Merida, the capital of Yucatan, Mexico (19°30′ and 21°35′ N in latitude, and 87°30′ 90°24′ W in longitude). The climate in the region is tropical (Aw) with an average annual temperature of 24-28°C and a range of total annual rainfall of 400-2000 mm [32]. Samples were obtained during the month of July 2017 and during June-July 2018. A cross-sectional study was carried out by collecting sand samples from 68 playground sandboxes in public parks of Merida; from each park, 20 gr of sand were obtained, 10 gr from the superficial region (< 2 cm) and 10 gr from a deeper region (2-10 cm or until reaching rock bottom). Each sample was divided in two 5 gr samples, resulting in 4 subsamples from each park; samples were placed in sterile tubes of 50 mL.

2.2. Extraction and Purification of Sand Samples. Extraction was performed by following the methodology described by Lélu *et al.* [33]; briefly, to each 5 gr sample, 10 mL of deionized water was added; posteriorly, it was mixed for 1 minute using a vortex mixer. Then, 20 mL of Sheather's sugar solution (specific gravity: 1.2) was added and centrifuged at 1500 g for 20 minutes; the obtained interface (13 mL) was transferred to another tube in which 35 mL of deionized water was added and then centrifuged at 1500 g for 20 minutes. From each tube, 1 mL of sediment was collected and placed in a 1.7 mL Eppendorf tube; this was centrifuged at 1500 g for 5 minutes and, then, the supernatant was eliminated (approximately 600 μL), and the remaining sediment from each vial was then combined in one tube, resulting in one tube for each sample (superficial and deep) from their respective parks. Purification was made by using the NucleoSpin® TriPrep (MACHERY-NAGEL, Germany) kit and following its manufacturer recommendations.

2.3. Nested PCR for the Detection of T. Gondii DNA. The nested PCR (nPCR) was used to amplify a fragment of 390 pb of SAG1 gene (main surface protein of *T. gondii*), using a thermocycler Veriti 96 wells (Applied Biosystems™). The first amplification was performed with the external primers sense 5′-GTTCTAACCACGCACCCTGAG-3′ and antisense 5′- AAGAGTGGGCTCTGTGA - 3′; in the

second amplification, primers used were internal sense 5′- CAATGTGCACCTGTAGGAAGC-3′ and internal antisense 5′-GTGGTTCTCCGTCGGTGTGAG-3′ [34]. The first amplification reaction was performed with 1X PCR buffer (PROMEGA) at a concentration of 2mM of $MgCl^2$; 0.8mM of dNTPs; 0.5 uM for each primer; 1.5U Taq polymerase; and 2 μL of DNA sample in a final volume of 25 μL. The second run had the same conditions as the first PCR; only the concentration of primers was 0.3 uM and for the second run 2 μL PCR of the product from the first round was used. The PCR conditions in the first run were 95°C for 5 min, followed by 30 cycles at 94°C for 30 s, 55°C for 1 min, and 72°C for 2 min. In the second run, it was 95°C for 5 min, followed by 35 cycles at 94°C for 30 s, 60°C for 1 min, and 72°C for 1 min and 30 s. As a positive control, DNA from *T. gondii* tachyzoites ($1x10^2$) of reference strain of *Toxoplasma gondii* (RH) strain was used; as a negative control, a master mix without DNA was used. The amplification products (390 pb) were visualized on agarose gel 1.5%, stained with ethidium bromide (0.5 μg/mL) using a Gel Doc™ XR+ Gel Documentation System (Bio-Rad™).

2.4. PCR for the Detection of A. Caninum and T. Canis DNA. An endpoint PCR was used to amplify a fragment of 380 pb of ITS-2 gene from *Toxocara canis* with the primer sense Tcan1 5′AGTATGATGGGCGCGCCAAT-3′ and antisense NC2 5′-TTAGTTTCTTTTCCTCCGCT-3′ [35] and a fragment of 427 pb of rRNA-ITS2 gene from *Ancylostoma caninum* with the primer sense A.canF 5′-AGCATTAGGCTAACGCCCGA-3′ and antisense A.canR 5′-AACGAGTTTGCTGTCATTCAGTCC-3′ [36]. The conditions for the PCR reaction were buffer PCR GoTaq® Green 1X (PROMEGA) at a concentration of 3mM of $MgCl^2$; 0.8 mM of dNTPs; 0.5 uM for both primers (forward and reverse); 1.5U Taq polymerase; and 3μL of DNA sample in a final volume of 20 μL. The PCR conditions were 94°C for 10 min, followed by 30 cycles at 94°C for 30 s, 60°C for 30s, and 72°C for 7 min, using a thermocycler Veriti 96 wells (Applied Biosystems™). As positive controls, DNA from *T. canis* adult nematodes and DNA from *A. caninum* eggs obtained from naturally infected dogs were used; as a negative control, a master mix without DNA was used. The amplification products were visualized on agarose gel 1.5%, stained with ethidium bromide (0.5 μg/mL) using a Gel Doc™ XR+ Gel Documentation System (Bio-Rad™).

3. Results

Presence of *T. gondii* DNA was detected in 11.8% (8/68) of all the evaluated sandboxes (Figure 1). Of the positive samples, 4 were from the superficial region (50%) and 4 from the deep region (50%) (Figure 2). All samples were negative for *T. canis* and *A. caninum*.

4. Discussion

The results demonstrate the presence of *T. gondii* in the evaluated sand samples, which represents an important risk factor due to the quantity of people that visit those recreational sites.

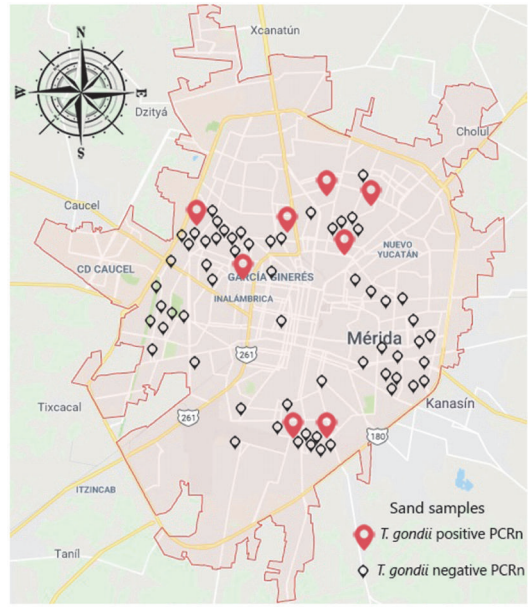

Figure 1: Distribution of sampled points of sand from sandbox playgrounds in the city of Merida, Yucatan, Mexico, where positive cases for *T. gondii* DNA were found through nested PCR.

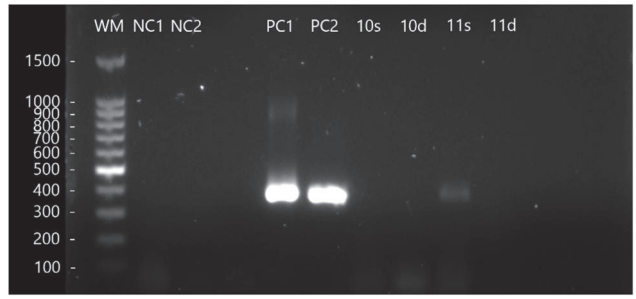

Figure 2: Agarose gel 1.5%. The well 11s (superficial) shows a positive amplification for *T. gondii*. WM: weight marker, NC1: negative control one, NC2: negative control two, PC1: positive control one ($1x10^2$ tachyzoites/mL), PC2: positive control two ($1x10^3$ tachyzoites/mL).

Results are similar to those reported by Wang et al. in China where they reported that 34 out of 268 (12.69%) soil samples were *T. gondii* positive [10], but lower than those reported by Gotteland et al. [11] in France where 71 out of 243 (29.2%) samples were positive; the difference is that this French village is mostly inhabited and cat populations concentrate which could explain the greater number of positive samples. In the studied region, the diversity of people that visit these places has been reported; from the interviewed people, it was found that 47% of adults visit the parks, 77.8% of interviewed teenagers visit the parks, and 71% of interviewed children also visit the parks [37]. Children represent the principal group at risk for acquiring the infection because of their behavior in playgrounds. In Mexico, seroprevalence of infection has been reported in this age group, with results of 10.4% in children <5 years old, 26.2% in 5-9-year-olds, and 28% in

10-14-year-olds [38]; therefore, it is clear that people are getting infected from an early age and the contact with contaminated soil could be one of the sources of infection, especially in children [39]. Toxoplasmosis is an infection that can cause severe lesions and even death in susceptible groups like people with AIDS and pregnant woman and their products of conception [40]. This infection is considered endemic in the study region, where prevalence of 20-90% has been reported in different population groups [28, 41]. Also, prevalence of *T. gondii* infection in people with AIDS and women with abortions has been reported as 47 and 59%, respectively [42–44], reiterating the impact that this parasite is represented in endemic regions.

The study region has favorable environmental conditions for the viability of *T. gondii* oocysts which is characterized by high temperatures (average: 23.5-30.7°C) and high humidity [32]; under this conditions, oocyst can survive for up to 21 months [7] which highlights its importance as a source of infection for people who come into contact with the infective form. In the same way, in this region, there is a high number of freely roaming cats, which are responsible for the environmental contamination. Cats can excrete up to 10 million oocysts a day and they do it for 7 to 20 days after the primary infection [6], to later develop immunity and stop excreting them. However, experimental studies have shown that they can reeliminate oocysts after a secondary infection [45]. In this region, a total of 433 public parks are present, occupying a total area of 2,329 860 m^2 (2.32 km^2) [46]. Some of the characteristics shared by the studied parks are the absence of barriers that prevent access to playgrounds (site of indiscriminate defecation) and the presence of stray animals (dogs and cats) in the parks, which means that any of these parks could be a potential site of defecation for these animals, especially cats, which have the characteristic of burying their feces in particulate and shallow materials such as sand and soil; it has been observed that they develop this habit even without having seen their mother do it [47]; therefore, it is clear that the sand areas of the parks are an ideal place for them to carry out this activity. In addition, in this region, there are no obligatory programs to control the overpopulation of stray cats, which contributes significantly to the maintenance of the contamination problem. Nevertheless, there are other strategies that could reduce this contamination; one of them would be to replace the sand pits of the parks by other materials like rubber floor, which softens falls and is antiskid and highly resistant to water making it ideal for outdoors, which would also prevent cats from excreting their feces in these areas. It is also important to educate the population, especially children, in the implementation of hygiene measures such as the constant washing of the hands after attending these recreational areas and taking care of their behavior to avoid the accidental ingestion of sand. The spatial distribution of the positive samples could be explained by the cat density which is correlated with human population density and food abundance [48]; the city of Merida has an average human population density of 38.02 per km^2; nevertheless, the highest density is reported in the south followed by the north part of the city, while the west and the east have lower densities [49].

However, it is worth mentioning that all the evaluated sand samples were negative for the presence of *T. canis* and *A. caninum*. This could be due to the behavior of dogs, which unlike cats, seem to have no predilection for a particular type of surface [50]. Despite the negativity in the samples, in the region of study, there has been reported a seroprevalence of *T. canis* of 29.2% in the human population [28]. On the other hand, there are no available reports of *A. caninum* in the human population. However, a 10% positivity was recently reported for *A. caninum* and 1% for *T. canis* in stool samples collected in public parks in the same study region [51]. In addition to *T. gondii*, *T. canis,* and *A. caninum*, the presence of cats and stray dogs circling around public parks represents a source of infection of various diseases such as salmonellosis, campylobacteriosis, leptospirosis, brucellosis, ehrlichiosis, cryptosporidiosis, giardiasis, and leishmaniasis [1, 2]. According to the Center for Disease Control and Prevention, toxoplasmosis and toxocariasis are infections considered as neglected diseases, because they are given little attention in their surveillance, prevention, and treatment, although they are affecting a large number of people with severe consequences in their health [52].

5. Conclusion

This is the first report of the presence of *T. gondii* DNA in playground sandboxes samples of the parks of the city of Merida, Yucatan, Mexico, which could represent an important source of infection for the people who visit these areas, especially for children. Control measures should be implemented to reduce the risk of infection in playground areas from parks located in Neotropical areas in Mexico, where the infection caused by *T. gondii* is not considered relevant.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors gratefully thank the students of the Colorado State University for their participation in carrying out the samplings.

References

[1] J. Jacob and B. Lorber, "Diseases transmitted by man's best friend: the dog," *Microbiology Spectrum*, vol. 3, no. 4, pp. 111–131, 2015.

[2] E. J. Goldstein and F. M. Abrahamian, "Diseases transmitted by cats," *Microbiology Spectrum*, vol. 3, no. 5, 2015.

[3] A. M. Tenter, A. R. Heckeroth, and L. M. Weiss, "*Toxoplasma gondii*: from animals to humans," *International Journal for Parasitology*, vol. 30, no. 12-13, pp. 1217–1258, 2000.

[4] G. Pappas, N. Roussos, and M. E. Falagas, "Toxoplasmosis snapshots: global status of toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis," *International Journal for Parasitology*, vol. 39, no. 12, pp. 1385–1394, 2009.

[5] J. P. Dubey and J. L. Jones, "Toxoplasma gondii infection in humans and animals in the United States," *International Journal for Parasitology*, vol. 38, no. 11, pp. 1257–1278, 2008.

[6] J. P. Dubey, D. S. Lindsay, and C. A. Speer, "Structures of Toxoplasma gondii tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts," *Clinical Microbiology Reviews*, vol. 11, no. 2, pp. 267–299, 1998.

[7] M. Lélu, I. Villena, M.-L. Dardé et al., "Quantitative estimation of the viability of Toxoplasma gondii oocysts in soil," *Applied and Environmental Microbiology*, vol. 78, no. 15, pp. 5127–5132, 2012.

[8] F. Robert-Gangneux and M. Dardé, "Epidemiology of and diagnostic strategies for toxoplasmosis," *Clinical Microbiology Reviews*, vol. 25, no. 2, pp. 264–296, 2012.

[9] A. J. C. Cook, R. E. Gilbert, W. Buffolano et al., "Sources of toxoplasma infection in pregnant women: European multicentre case-control study," *British Medical Journal*, vol. 321, no. 7254, pp. 142–147, 2000.

[10] M. Wang, P. Meng, Q. Ye et al., "Detection of Toxoplasma gondiioocysts in soils in Northwestern China using a new semi-nested PCR assay," *BMC Veterinary Research*, vol. 10, no. 238, 2014.

[11] C. Gotteland, E. Gilot-Fromont, D. Aubert et al., "Spatial distribution of Toxoplasma gondii oocysts in soil in a rural area: Influence of cats and land use," *Veterinary Parasitology*, vol. 205, no. 3-4, pp. 629–637, 2014.

[12] T. R. Santos, C. M. Nunes, M. C. Luvizotto et al., "Detection of Toxoplasma gondii oocysts in environmental samples from public schools," *Veterinary Parasitology*, vol. 171, no. 1-2, pp. 53–57, 2010.

[13] L. Doganci, M. Tanyuksel, E. Araz et al., "A probable outbreak of toxoplasmosis among boarding school students in Turkey," *Clinical Microbiology and Infection*, vol. 12, no. 7, pp. 672–674, 2006.

[14] E. L. Carmo, M. M. Póvoa, N. S. Monteiro et al., "Surto de toxoplasmose humana no distrito de monte dourado, município de almeirim, pará, brasil," *Revista Pan-Amazônica de Saúde*, vol. 1, pp. 61–66, 2010.

[15] S. M. Teutsch, D. D. Juranek, A. Sulzer, J. P. Dubey, and R. K. Sikes, "Epidemic toxoplasmosis associated with infected cats," *The New England Journal of Medicine*, vol. 300, no. 13, pp. 695–699, 1979.

[16] D. Despommier, "Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects," *Clinical Microbiology Reviews*, vol. 16, no. 2, pp. 265–272, 2003.

[17] W. H. Roldán, Y. A. Cavero, Y. A. Espinoza, S. Jiménez, and C. A. Gutiérrez, "Human toxocariasis: a seroepidemiological survey in the amazonian city of yurimaguas, Peru," *Revista do Instituto de Medicina Tropical de São Paulo*, vol. 52, no. 1, pp. 37–42, 2010.

[18] S. L. McGuinness and K. Leder, "Global burden of toxocariasis: a common neglected infection of poverty," *Current Tropical Medicine Reports*, vol. 1, pp. 52–61, 2014.

[19] N. N. Cortés, C. R. Núñez, B. G. L. Guiliana, P. A. H. García, and R. H. Cárdenas, "Presence of anti-toxocara canis antibodies and risk factors in children from the Amecameca and chalco regions of México," *BMC Pediatrics*, vol. 15, no. 1, article 65, 2015.

[20] C. Romero Núñez, G. D. Mendoza Martínez, S. Yañez Arteaga, M. Ponce Macotela, P. Bustamante Montes, and N. Ramírez Durán, "Prevalence and risk factors associated with toxocara canis infection in children," *The Scientific World Journal*, vol. 2013, Article ID 572089, 4 pages, 2013.

[21] D. Otero, A. M. Alho, R. Nijsse, J. Roelfsema, P. Overgaauw, and L. Madeira de Carvalho, "Environmental contamination with toxocara spp. eggs in public parks and playground sandpits of greater lisbon, Portugal," *Journal of Infection and Public Health*, vol. 11, pp. 94–98, 2018.

[22] H. Mizgajska, "Eggs of Toxocara spp. in the environment and their public health implications," *Journal of Helminthology*, vol. 75, no. 2, pp. 147–151, 2001.

[23] P. O'Lorcain, "Prevalence of Toxocara canis ova in public playgrounds in the Dublin area of Ireland," *Journal of Helminthology*, vol. 68, no. 3, pp. 237–241, 1994.

[24] C. Romero, A. García, G. D. Mendoza Martínez et al., "Contamination for Toxocara spp. in Tulyehualco parks, Mexico," *Revista Científica de Veterinaria*, vol. 19, pp. 253–256, 2009.

[25] A. Loukas, P. J. Hotez, D. Diemert et al., "Hookworm infection," *Nature Reviews Disease Primers*, vol. 2, article 16088, 2016.

[26] P. Prociv and J. Croese, "Human eosinophilic enteritis caused by dog hookworm Ancylostoma caninum," *The Lancet*, vol. 335, no. 8701, pp. 1299–1302, 1990.

[27] D. Traversa, A. F. Di Regalbono, A. Di Cesare, F. La Torre, J. Drake, and M. Pietrobelli, "Environmental contamination by canine geohelminths," *Parasites & Vectors*, vol. 7, no. 1, article 67, 2014.

[28] A. Ortega-Pacheco, J. F. J. Torres-Acosta, A. Alzina-López et al., "Parasitic zoonoses in humans and their dogs from a rural community of tropical Mexico," *Journal of Tropical Medicine*, vol. 2015, Article ID 481086, 6 pages, 2015.

[29] G. R. Cortez-Aguirre, M. Jiménez-Coello, E. Gutiérrez-Blanco, and A. Ortega-Pacheco, "Stray dog population in a city of Southern Mexico and its impact on the contamination of public areas," *Veterinary Medicine international*, vol. 2018, Article ID 2381583, 6 pages, 2018.

[30] R. I. Rodríguez-Vivas, E. Gutierrez-Ruiz, M. E. Bolio-González et al., "An epidemiological study of intestinal parasites of dogs from Yucatan, Mexico, and their risk to public health," *Vector-Borne and Zoonotic Diseases*, vol. 11, no. 8, pp. 1141–1144, 2011.

[31] I. B. Hernandez-Cortazar, K. Y. Acosta-Viana, E. Guzman-Marin, A. Ortega-Pacheco, J. C. Segura-Correa, and M. Jimenez-Coello, "Presence of toxoplasma gondii in drinking water from an endemic region in Southern Mexico," *Foodborne Pathogens and Disease*, vol. 14, no. 5, pp. 288–292, 2017.

[32] INEGI, "Anuario estadístico y geográfico por entidad federativa," México, 2017.

[33] M. Lélu, E. Gilot-Fromont, D. Aubert et al., "Development of a sensitive method for Toxoplasma gondii oocyst extraction in soil," *Veterinary Parasitology*, vol. 183, no. 1-2, pp. 59–67, 2011.

[34] C. Su, E. K. Shwab, P. Zhou, X. Q. Zhu, and J. P. Dubey, "Moving towards an integrated approach to molecular detection and identification of Toxoplasma gondii," *Parasitology*, vol. 137, no. 1, pp. 1–11, 2010.

[35] D. E. Jacobs, X. Zhu, R. B. Gasser, and N. B. Chilton, "PCR-based methods for identification of potentially zoonotic ascaridoid

parasites of the dog, fox and cat," *Acta Tropica*, vol. 68, no. 2, pp. 191–200, 1997.

[36] W. Hu, S. Wu, X. Yu et al., "A multiplex PCR for simultaneous detection of three zoonotic parasites ancylostoma ceylanicum, A. caninum, and giardia lamblia assemblage A," *BioMed Research International*, vol. 2015, Article ID 406168, 6 pages, 2015.

[37] S. Pérez Medina and L. F. Fargher, "Use of recreational parks in Mérida, Yucatán," *Estudios Demográficos y Urbanos*, vol. 31, no. 3, pp. 775–810, 2016.

[38] O. Velasco-Castrejón, B. Salvatierra-Izaba, J. Vardespino et al., "Seroepidemiología de la toxoplasmosis en México," *Salud Pública México*, vol. 34, pp. 222–229, 1992.

[39] Q. F. Meng, H. L. You, N. Zhou, W. L. Dong, W. L. Wang, and W. Cong, "Seroprevalence of Toxoplasma gondii antibodies and associated risk factors among children in Shandong and Jilin provinces, China," *International Journal of Infectious Diseases*, vol. 30, pp. 33–35, 2015.

[40] J. G. Montoya and O. Liesenfeld, "Toxoplasmosis," *The Lancet*, vol. 363, no. 9425, pp. 1965–1976, 2004.

[41] I. Hernández-Cortazar, K. Y. Acosta-Viana, A. Ortega-Pacheco et al., "Toxoplasmosis in mexico: epidemiological situation in humans and animals," *Revista do Instituto de Medicina Tropical de São Paulo*, vol. 57, no. 2, pp. 93–103, 2015.

[42] R. Góngora-Biachi, P. González-Martínez, C. Castro-Sansores et al., "Anticuerpos contra Toxoplasma gondii en pacientes con VIH en Yucatán," *Revista de Investigacion Clinica*, vol. 50, pp. 419–422, 1998.

[43] I. A. Vado-Solís, V. Suárez-Solís, B. Jiménez-Delgadillo, J. E. Zavala-Velázquez, and J. C. Segura-Correa, "Toxoplasma gondii presence in women with spontaneous abortion in Yucatan, Mexico," *The Journal of Parasitology*, vol. 99, no. 2, pp. 383–385, 2013.

[44] I. B. Hernández-Cortazar, K. Y. Acosta-Viana, E. Guzman-Marin et al., "Toxoplasma gondii in women with recent abortion from Southern Mexico," *Asian Pacific Journal of Tropical Disease*, vol. 6, no. 3, pp. 193–198, 2016.

[45] J. P. Dubey, "Duration of immunity to shedding of Toxoplasma gondii oocysts by cats," *Journal of Parasitology*, vol. 81, no. 3, pp. 410–415, 1995.

[46] Ayuntamiento de Mérida: Instituto Municipal de Planeación, "Sistema de Gestión de Espacios Públicos, (2018) 25–41," http://isla.merida.gob.mx/serviciosinternet/ordenamientoterritorial/docs/SistemaGestion.pdf.

[47] P. L. Borchelt, "Cat elimination behavior problems," *Veterinary Clinics of North America: Small Animal Practice*, vol. 21, no. 2, pp. 257–264, 1991.

[48] G. Aguilar, M. Farnworth, and L. Winder, "Mapping the stray domestic cat (Felis catus) population in New Zealand: species distribution modelling with a climate change scenario and implications for protected areas," *Applied Geography*, vol. 63, pp. 146–154, 2015.

[49] Ayuntamiento de Mérida, "Programa Municipal de Desarrollo Urbano de Mérida, 2018," http://isla.merida.gob.mx/servicios-internet/ordenamientoterritorial/paginas/pmdu.phpx.

[50] A. Beck, *The Ecology of Stray Dogs*, NotaBell Books, West Lafayette, Indiana, 1st edition, 2002.

[51] R. A. Medina-Pinto, R. I. Rodríguez-Vivas, and M. E. Bolio-González, "Zoonotic intestinal nematodes in dogs from public parks in Yucatán, México," *Biomédica*, vol. 38, no. 1, pp. 105–110, 2018.

[52] Centers for Disease Control and Prevention, "Parasites-Neglected Parasitic Infections (NPIs), 2017," https://www.cdc.gov/parasites/npi/index.html.