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Research Article

Presence of Zoonotic Helminths in Dog Feces and Soil in the Chapultepec Forest of Mexico City

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Abstract

Some intestinal dog parasites represent a worldwide problem for human health due to their biotic and zoonotic potential. Metropolitan areas with high concentrations of dogs can be a health risk because of soil contamination with fecal matter. The objective of this study was to determine the presence of zoonotic intestinal parasites in dog feces and soil in the Chapultepec Forest of Mexico City. 210 fecal samples were examined by using the flotation method and direct smear stained with Zhiel Neelsen. Simultaneously, 150 soil samples were studied by means of the Ferreira method. The results were analyzed by using SPSS, version 21, and a chi-square test. The prevalence of fecal parasites was 51.5%. The prevalences of zoonotic parasites were: *T. canis* 34.8%, *A. caninum* 16.7%, and *Cryptosporidium* spp. 2.4%. The prevalence of eggs of *T. canis* in soil samples was 9.3%. Contamination of the forest surface with infectious forms of zoonotic parasites can be a source of infection for visitors, especially children. It is important to implement information and prevention campaigns for zoonotic infection control through health education of the population, in general, and of pet owners, in particular, as well as canine population control.

Key words: zoonoses, dogs, environmental contamination, soils, parks.

Introduction

Dogs are considered to be reservoirs, dissemination agents, and infection sources of a large number of infectious agents [1,2]. According to the World Health Organization (WHO), a dog can transmit to a human up to 53 zoonotic diseases caused by viruses, bacteria, fungi, and parasites of medical and veterinary importance. Among these infectious agents, canine intestinal helminths and protozoos stand out. Therefore, dogs are considered to be the main responsible for environmental contamination with fecal matter of forests, parks, gardens, and sidewalks of public spaces in rural and urban areas. [3-5].

Canine intestinal parasites represent a problem for human health due to their biotic and zoonotic potential, since people who live in urban areas such as large cities and megapolises are exposed to canine zoonotic parasite infections. [1,2]. In these areas of human concentration, forests, parks and green areas are an ideal place of recreation for inhabitants, but at the same time a danger of being infected with parasitic zoonoses transmitted by dogs. They represent an increasing risk because of the presence of these animals in recreation sites who are homeless or with an owner and who daily defecate in these public spaces [6, 2].

Different epidemiological studies conducted in developed and developing countries in both rural and urban areas report the presence of eggs, larvae, cysts and oocysts of canine intestinal parasites in the samples of contaminated soil obtained from playgrounds, parks, and gardens. Hence, soil is considered a way of transmitting different etiological agents that cause canine zoonotic disease [7, 4, 8].

Profuse environment fecal contamination with eggs, larvae, cysts and oocysts excreted by infected dogs boosts the transmission of zoonoses. Different parasite infectious forms can survive and remain viable in the environment over a long period of time. Thus, there is an increasing risk of developing cryptosporidiosis or *visceral* and *ocular migrans* larva syndromes caused by *Toxocara canis* or *migrans cutaneous* larva produced by *Ancylostoma caninum* and *Ancylostoma brasiliensis* as well as other diseases produced by helminths transmitted through a direct contact with contaminated soil [4, 9-12]. The Chapultepec Forest of Mexico City is an eminent place for family recreation that does not avoid this situation. There live stray dogs that have found in it an ideal place for reproduction, in this way increasing canine population. In addition, many pets are brought to this place who defecate there contaminating the soil, and this a fundamental epidemiological factor in transmitting zoonotic parasites that infect the human population who daily go to this site [13, 5, 14].

Objective

This work was carried out in order to determine the presence of eggs of helminths and oocysts of canine zoonotic parasites in the soil and fecal matter samples collected in the Chapultepec Forest, Mexico City.

Material and methods

Study design

In the first semester of 2015, descriptive cross-section exploratory sampling was performed with the aim of detecting canine zoonotic enteroparasites in the soil samples and fecal matter collected in the Chapultepec Forest of Mexico City.

Study area

The Chapultepec Forest is in Mexico City that has 8,851,080 inhabitants and is located at parallels 19° 36' and 19° 03' north of the equator, and at 98° 57' and 99° 22' west of the Greenwich Meridian, at an altitude 2,240 meters (7,350 ft), with humid temperate climate and average annual temperature 16° C. The city consists of 16 administrative delegations distributed on the territory of 1,485 square kilometers (573 sq mi). The Chapultepec Forest is an urban park located in the borough (delegación) of Miguel Hidalgo, and is one of the largest of its type in the Western Hemisphere with the land area of 677 hectares. It is divided into three sections. The first one occupies 274 hectares, 182 of which correspond to green areas; there are two lakes. The second section area is 160 hectares. The third section has the total area of 243 hectares, 53.5 hectares of which belong to green areas [15].

Universe and Samples

Collection of soil samples

Samples were obtained through a simple random method. In each of the three forest sections, 50 soil samples were collected, preferably before 12 p.m. in shaded areas of family recreation. Initially, the topsoil was removed with a metal shovel. Each sample was obtained from a 10 \times 20 \times 2 cm surface area with a soil mass of approximately 200 grams. The obtained samples were deposited in polyethylene bags with the data corresponding to each collection section. All the samples were kept cool at 4° C before being analyzed.

The soil analysis was conducted through the Ferreira method. Glass beakers were used to deposit samples separately by homogenizing them with 250 ml of distilled water. The homogenates were sieved using cotton gauze and were collected in Ferreira tubes [16]. The tubes were centrifuged at 2,000 revolutions per minute for three minutes, discarding the floating material. The sediments were resuspended in 35 ml of zinc sulfate at a density of 1: 200; the suspension was homogenized by a second centrifugation at 2000 rpm during five minutes. The floating material was treated through fresh preparations with Lugol solution. The observation was performed by using Carl Zeiss brightfield microscopes at 100 and 400 magnifications. The identification of parasite forms was fulfilled according to their morphology.

Collection of fecal samples

In three sections of the forest, 210 samples of fecal matter were collected into polyethylene containers with a hermetic lid that were marked with the corresponding section and were placed in a cold net for their transfer to the laboratory of Medical Parasitology of the Metropolitan Autonomous University (Universidad Autónoma Metropolitana), Campus Xochimilco, where they were kept cool at 4° C until the moment of their study.

Medical tests

Each sample was examined macroscopically to detect adult worms and/or cestode proglottids. The identification of oocysts of *Cryptosporidium* spp. was carried out by a direct fecal spread stained with modified Ziehl Neelsen [17]. Simultaneously, the stool ova and parasite exam was fulfilled through the floatation-concentration technique with zinc sulfate at 1:18 [18]; the floating material was treated through fresh preparations with Lugol solution. The observation was performed by using photonic microscopy through Carl Zeiss brightfield microscopes at 100, 400 and 1000 magnifications. The identification of parasitic forms was done according to their staining affinity and morphology. In the case of detecting ancylostomatidae eggs, the morphological identification was carried out by using a stool culture through the Harada-Mori method, and by observing the larva characteristics in order to identify the species.

Statistical analysis

The sampling information was organized and coded by using SPSS for Windows, version 21.0 (SPSS Inc., Chicago IL, USA). The data obtained from the variables such as the presence of *Cryptosporidium* spp. and other enteroparasites in three sections of the Chapultepec Forest was used to construct association relationships, graphs, and contingency tables that allowed to have a quantitative description of the collected samples. Chi-square statistical tests and the Fisher exact test were applied to determine the association among the variables with the significance level of 0.05%.

Results

The study consisted in analyzing 210 fecal samples collected in three forest sections (68 in the first section, 62 in the second one, and 80 in the third one) as well as 50 soil samples from each of three sections. Table 1 shows the general prevalence of 51.5% of helminth eggs found in 210 fecal samples, the infection by *T. canis* was present in 34.8% of them, and by *A. caninum* in 16.7%. Table 2 displays the general prevalence of helminth eggs that were present in the fecal samples collected in each of three sections of the forest. In the first

section, the eggs of *T. canis* prevailed and in the third section the eggs of *A. caninum*. Table 3 shows that the general analysis of the results of the medical tests in three sections of the forest reveals the presence of helminth eggs in these areas with an estimated p of < 0.001. Through the microscopic analysis of 210 fecal samples stained by using the Zhiel Neelsen technique, *Cryptosporidium* spp, oocysts were spotted in five samples, i.e. in 2.4% of all samples. Of 150 soil samples analyzed through the Ferreira method, in 14 (9.3%), *T.* canis eggs were found, and free-living larvae were observed in 12 samples (7.3%).

Table 1. General prevelance of helminth eggs in 210 fecal samples obtained in the Capultepec Forest, Mexico

Eggs	Frequency	Porcentage	
	35	16.7	
T. canis	73	34.8	
Total	210	100.0	
Negative	102	51,5	

Table 2. Prevalence of canine helminth eggs in 210 feces analyzed by Faust's method in three section of the Chapultepec Forest, Mexico City

Helminth eggs	Section			Total
	1	2	3	Totai
A. caninum	10	10	15	35
T. canis	32	28	13	73
Negative	26	24	52	102
Total	68	62	80	210

 Table 3. General prevalence of canine helminth eggs in 210 feces analyzed by Faust's method in three section of the Chapultepec Forest, Mexico City

Negative		Faust's method		
		Negative	Positive	Total
Section	1	26	42	68
	2	24	38	62
	3	52	28	80
Total		102	108	210

* p < 0.001

Discussion

The Chapultepec Forest, the oldest urban park in the Americas and one of the oldest in the world, has been visited by 15 million people annually and by 200,000 every weekend. It is one of the largest urban parks in the world, on a par with Central Park in NY, Hyde Park in London, or Le Bois de Boulogne in Paris. The forest green areas are a favorite place for city resident recreation. However, a large number of homeless dogs that live there present a danger of physical aggression toward the visitors and also a risk to them of getting different zoonotic diseases such as rabies, syphilis; the stray canine population as well as parasitized dogs that accompany visitors indiscriminately contaminate the soil with helminth eggs and zoonotic protozoan cysts that are present in their feces, and in this way the contaminated soil represents an important source of infection and a significant impact on public health [6, 7]. The species of zoonotic nematodes identified in this study were T. canis and A. caninum. The first type is considered to be the main etiological agent of toxocariasis, a parasitic zoonosis that causes significant morbidity and a serious public health problem in the world, mainly in developing countries [7]. The morbidity of human toxocariasis is related to the following factors: a close contact of humans with dogs and cats, an increase of the dog and cat population, a shortage of sanitary control of the fecal matter evacuated by these animals both in public sites and at home, scarce knowledge that people have of this zoonosis, and, especially, a lack of a timely diagnosis that could prevent the development and evolution of the disease before it could produce cases as serious as loss of vision or death [12].

In this study, the rate of soil contamination with this zoonosis in the Chapultepec Forest was 9.3%. Similar studies of soil contamination with *T. canis* eggs in different countries reported frequencies similar to those obtained by us, for example: 6.8% in Poland [14] 16.4% in Madrid, Spain [16]; 6.6% in Italy [20], and 9.2% in Chile [21]. However, some developing countries have reported frequencies that are higher than those obtained in our study, such as 20.3% in Iran [10], or the one in a study of 25 state parks in Presidente Prudente, Brazil where *T. canis* was reported in 96.6% of sampled parks [22].

From the epidemiological point of view, the biotic potential of *T. canis* is enormous because a female can produce up to 200,000 eggs per day. The Pan American Health Organization (PAHO) has estimated that 1 gram of dog fecal matter can contain up to 15,000 *Toxocara* eggs that are deposited in the ground by walking people, rain, wind or vectors, [23, 24]. The thick layer of *Toxocara* eggs makes them resistant to cold and environmental changes; therefore, under adequate humidity and temperature conditions, they can survive in the earth surface for several years. Thus, despite the fact that the soil may appear to be clean because the feces were collected by the health personnel or because the fecal material has disintegrated and there is no odor, it can be contaminated with microscopic infectious forms of parasites.

Soil contaminated with *T. canis* larvae is the main source of infection for humans. In humans as well as in dogs, the infecting form is a larval egg. *T. canis* larval eggs affect different organs in both humans and dogs; however, adult parasites develop only in dogs. Thus, people who visit recreation sites with contaminated soil are exposed to infection. The most vulnerable people are those under eight years old because their play activities put them in greater contact with soil. Infection in children is caused mainly by geophagy or ingestion of *T. canis* infectious larvae from contaminated soil, or ingestion of larval eggs that are present in dogs' hair [25-28]. In developed countries, despite the fact that the prevalence of parasitic diseases is usually low there, toxocariasis is the most common helminth disease [3, 29].

The ingested eggs hatch in the digestive tract, and the larvae enter the intestinal mucosa, then reach mesenteric vessels and spread to different organs and tissues where the host's response traps them by producing granulomas, whose degree of disease will be in direct proportion to the number of infected ingested larval eggs. In the clinical form of the disease known as visceral migrant larva, the symptoms are nonspecific and consist of abdominal pain, anorexia, malaise, behavioral disorders, cervical adenitis, hepatomegaly, pain in extremities, and fever. Children under 5 years old are the most affected, and the main risk factors in this population are geophagy and close contact with dogs. The most severe form of the disease occurs in the eyes, where it produces ocular migrant larva syndrome (LMO), [30]. It has been reported that a single larva can cause unilateral blindness. Vision loss occurs because of the acute inflammatory reaction of the retina and optic nerve. The risk of being in contact with soils contaminated with *T. canis* eggs is very severe because a case of congenital ocular toxocariasis in a newborn has been recently documented [31].

In the case of *A. caninum*, human infection is caused by penetration of a filariform larva into the skin, but as it is unable to invade deeper tissues, it produces a skin lesion that is several centimeters long and is very itchy due to carving a tunnel in the epidermis known as migratory cutaneous larva or verminous creeping dermatitis [11, 32, 33].

The prevalence rates of this helminth reported by some authors of similar studies vary from 4.2% in Chile [21] 6.8% in Italy [20], 13.5% in Brazil [34]. A clear example of the severity of soil contamination with geohelminths of canine origin is beach sand in Brazil where the presence of *A. caninum* in 82.5% of its samples was reported [35].

The detection of *Cryptosporidium* spp. in fecal samples of examined dogs supports the idea that infected canines are a potential source of human infection [36]. Dog infection with *Cryptosporidium* spp. reported in this study is less than that obtained in the studies with similar characteristics performed in France 2.6% [37], Sao Paulo, Brazil, 3.1% [38], and is similar to infection reported in Zaragoza, Spain, 5.5% [39], Egypto 5.4% [40] Niagara, Canada, 7.4% [41] Prague, Czech Republic, 9.3%; Japan, 7.2% [42], and the Netherlands, 8.7% [25]. However, the infection data obtained in the present study were less than 40% reported in the city of Campos dos Goytacases, Brazil [43].

The discrepancy in the above-mentioned results is due to different factors such as climatic conditions of the place of study, soil characteristics, soil contamination degree, sociocultural factors, and laboratory procedures.

The presence of *Cryptosporidium* spp. in infected animal feces plays an important role in transmitting these microorganisms, since dogs constantly eliminate a small number of sporulated oocysts that is, however, sufficient to infect humans and other animals. The problem gets worse if we consider the concatenation of some biological and climatic factors inherent to the parasite such as low dosage of oocysts (10 oocysts) required to develop infection [44]. The resistance of oocysts to environmental changes as well as to the conventional water treatment becomes epidemiologically important given that forest and garden irrigation is generally performed with water which contributes to soil contamination and oocyst dissemination and viability. In damp environments such as public parks, *C. parvum* oocysts can remain viable for 2-6 months [45, 40, 11].

Another factor that contributes to dogs' infection with *Cryptosporidum* is the biological cycle of the latter that consists of three autoinfectious reproductive phases as well as the production of thin-walled oocysts that embed themselves deeply in the host intestine originating chronic infections without a need of new infections. As a result of this autoinfection, during defecation a dog releases constant

infectious oocysts that contaminate soil as in the case of experimentally infected dogs who had up to 10,000 oocysts per one gram of their fecal material [46].

It is necessary to advise people with high risk such as immunocompromised or of extreme ages of the life and who visit parks to maximize their hygienic habits as, for example, washing hands after touching the hair of the dogs that go to these places regardless of whether they are stray or with an owner [28].

Despite a small number of samples, the rates of infection with zoonotic enteroparasites detected in the present work are important from the epidemiological and public health point of view because of soil contamination by enteric zoonotic species of parasitized animals that live or are brought to parks with ecological characteristics similar to those of the Chapultepec Forest in Mexico City [47-49].

With the aim of preventing zoonotic diseases transmitted by dogs, it is vital to implement actions such as daily collection of fecal matter in recreation areas; the control of synanthropic flies that participate in the dissemination of different zoonoses [23, 24], the direct participation of dog owners that visit the forest in collecting and eliminating stool evacuated by their pets; promotion of semi-annual de-worming; and scheduled sterilization in order to contribute to the reduction of street canine population, since many puppies are released to streets at an early age. Implementing these actions, undoubtedly, will help to get within a short time healthier environment for the inhabitants of Mexico City [50].

Finally, we believe that it is necessary to conduct more studies in order to detect the presence of intestinal parasites in animals as family members and, thereby, to evaluate the impact that these agents have on human health. In addition, such studies will provide the basis for recommending control measures in health programs.

Conclusions

The results of the study highlight the risk of transmitting canine zoonotic parasitosis to the human population, especially children, during their public park visits.

The presence of canine fecal matter in urban area soil is the main source of enteric pathogen infection of stray and domestic animals as well as humans. Preventing soil contamination is the most important way in the prevention of zoonoses transmitted by dogs together with other actions such as social responsibility of a strict control of canine fecal matter elimination not only in the Chapultepec Forest, but also in Mexico City streets, gardens and parks that include dog owners' participation and health-authorities' participation in the case of stray dogs. In addition to this, it is crucial to encourage quarterly de-worming of captured dogs, especially those under one-year-old. Through these simple measures, in a short term it would be possible to reduce soil contamination and minimize human and animal health risk.

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