INFECTIONS AND RISK FACTORS WITH ZOONOTIC INTESTINAL HELMINTHS IN PUPPIES ATTENDING FOR GOVERNMENTAL VETERINARY SERVICES IN LIBERTADOR MUNICIPALITY, CARACAS, VENEZUELA

Infecciones y factores de riesgo con helmintos intestinales zoonóticos en cachorros atendidos por servicios veterinarios gubernamentales en el municipio Libertador, Caracas, Venezuela

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ABSTRACT

Intestinal helminths of dogs are pathogens with zoonotic potential for humans. Dogs can be effectively treated by anthelmintic drugs, but the control of parasitic diseases may be difficult in those countries that are suffering an economic crisis. The aim of the study was to assess the prevalence of zoonotic intestinal helminths in puppies attending to governmental veterinary services in Libertador Municipality, Caracas, Venezuela, and the risk factors associated to the parasitic infections. A questionnaire was designed to collect information about the dog and their owner, dog management practices and to assess owners' awareness about the risks of parasites for animal and human health. Fecal samples obtained from the puppies between 1-6 months old (*n* = 272) were analyzed by microscopy after flotation technique with saturated sucrose solution. Associations between parasitism, host and management practices were quantified by univariate analyses and odds ratios. Helminths eggs were found in 66.3 % of the samples (180/272); *Toxocara canis*, *Dipyllidium caninum* and *Ancylostoma* spp. in 45.2; 11.8 and 5.5 %, respectively. Of 82.5% of the puppies restricted at home, 66.82% were parasitized. Female owner, frequency of cleaning of feces and knowledge about zoonoses caused by dog parasites constituted risk factors by univariate analysis. Due to the high level of infected puppies and that most of the owners ignore about zoonotic risks of parasites, dogs can be a major source to human infections in Libertador Municipality in Caracas.

Key words: Helminths; dogs; risk factors; Venezuela; public veterinary services

RESUMEN

Los helmintos intestinales de los perros son patógenos con potencial zoonótico para los humanos. Esas infecciones pueden tratarse eficazmente con antihelmínticos, pero en países que sufren crisis económicas, el control de las enfermedades parasitarias puede ser difícil. El objetivo del estudio fue evaluar la prevalencia de helmintos intestinales zoonóticos en cachorros que asistieron a servicios veterinarios gubernamentales en el municipio Libertador, Caracas, Venezuela y los factores de riesgo asociados a la parasitosis. Se diseñó un cuestionario para recopilar información sobre el perro y propietario, las prácticas de manejo y evaluar el conocimiento sobre los riesgos de los parásitos para la salud animal y humana. Se obtuvieron muestras de heces de cachorros entre 1 y 6 meses de edad (n = 272) y se analizaron mediante la técnica de flotación en solución hipersaturada de azúcar. Las asociaciones entre el parasitismo, el hospedador y las prácticas de manejo se cuantificaron mediante análisis univariables y odds ratio. Se observaron huevos de helmintos en 66,3% de las muestras (180/272); *Toxocara canis, Dipyllidium caninum* y *Ancylostoma* spp. en 45,2; 11,8 y 5,5%, respectivamente. Un 82,5% de los cachorros estaba restringido al hogar y de éstos, 66,82% estaban parasitados. El propietario de sexo femenino, la frecuencia de limpieza y el conocimiento sobre zoonosis causadas por los parásitos constituyeron factores de riesgo. Debido al alto nivel de cachorros infectados y a que la mayoría de los propietarios ignoran los riesgos zoonóticos se concluye que los cachorros pueden constituir una fuente importante para infecciones parasitarias humanas en el municipio Libertador de Caracas.

Palabras clave: Helmintos; caninos; factores de riesgo; Venezuela; servicios veterinarios públicos.

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INTRODUCTION

The role of dogs (*Canis lupus familiaris*) as sentient beings that guarantee physiological and psychological comfort to their owners is unquestionable. However, dogs may harbor many parasites potentially transmissible to humans which may represent a health risk especially to children, the elderly and the immunocompromised [23]. Human infections with canine intestinal worms are among the most common zoonotic infections. Pullan et al. [31] estimate worldwide by 2010, over 1.45 billion people were infected with at least one species of intestinal nematodes, most of the cases being concentrated in underdeveloped areas. Several enteric parasites have been recognized in dogs but not all have the potential for transmission to humans [8]. *Toxocara canis, Ancylostoma* spp. (*A. caninum, A. braziliense*), *Dipyllidium caninum* and *Echinococcus granulosus* are zoonotic parasites worldwide [2, 11, 22, 25, 43].

Despite advances in prophylaxis and treatment of parasitic diseases, these parasites are responsible for significant morbidity in both dogs and humans due to several and complex environmental, geographic, cultural and socioeconomic factors. Even now, zoonotic parasites constitute a challenge for both veterinarians and physicians in urban areas with a high population and closer interactions between dogs and humans [9, 19, 40]. Human toxocariasis is still considered a neglected disease as a result of the higher seroprevalences but a few resources dedicated to a preventable disease [41]. Infections with *Toxocara* spp. are a major health problem because larval migration results in a multisystemic disease that include visceral, neural and ocular symptoms [22]. Hookworms (*Ancylostoma* spp.) infection causes cutaneous larva migrans and eosinophilic enteritis [21]. Human infections with *D. caninum* are rare, but it is more likely to occur in young children who kiss or are licked by their infected pets. The patients with dipylidiasis are often asymptomatic but symptoms as anal pruritus, diarrhea, mild abdominal pain, decrease in appetite, indigestion, and gastrointestinal tract disturbances, may be seen [15].

The Metropolitan District of Caracas is divided into five Municipalities (Baruta, Chacao, Sucre, El Hatillo and Libertador). Libertador is one of the smallest Municipalities in Venezuela but is the largest in terms of population, with approximately 1,943,901 inhabitants in a total area of 433 km^2 [20]. Updated data on the number of dogs in the Municipality is absent; nonetheless, in 1989, the number of dogs was calculated in 286,079 representing 8% of the inhabitants of Metropolitan District of Caracas [30].
At the present time, there are two veterinary services at the Municipality, funded with governmental budget: Foundation for the protection of the fauna, “Funda Fauna” and “Misión Nevado”. Both veterinary services are located around lower-middle class neighborhoods and mainly aimed at those owners who cannot afford the costs of a private veterinary hospital.

A few reports concerning prevalence of gastrointestinal parasites in dogs from Venezuela include dogs under veterinary care in Maracaibo, Western Zulia State [33], from a rural locality in Western Falcon State [6] and dogs housed in animal facilities at Universidad Central in Maracay, Northern Venezuela [32]. Until now, no reports in dogs or the risk factors associated to zoonotic parasites in Caracas have been published. Additionally, the economic crisis in Venezuela has forced to abandon the pets due to elevated costs of food and treatments [12, 17]. Hence, the aim of the present study was to evaluate the prevalence of intestinal helminths in puppies attending for governmental veterinary services at Libertador Municipality in Caracas and to identify the risk factors associated to the parasitic infection.

MATERIALS AND METHODS

Study area

The study was carried out in Libertador Municipality (10°30′21″N 66°54′52″W) in City Caracas, at the veterinary centers of “Funda Fauna”: 23 de Enero at Western, (10°30′21″N 66°56′06″W), El Valle at Southern, (10°28′02″N 66°54′26″W) and the veterinary centers of “Misión Nevado”: San Agustín-Nuevo Circo at Eastern, (10°29′39″N 66°54′37″W) and San Bernardino at Northeastern (10°31’43”N 66°53’54”W).

Animals and samples

A total of 272 fecal samples were collected during the period July-September 2016. Each puppy between one to six months old which attended to veterinary centers of Funda Fauna (n = 248) was sampled; in the case of Misión Nevado, all the puppies housed in kennels to be given in adoption (n = 24) were sampled. Particular data of each dog (name, age, sex, breed, physical condition) were recorded.

Fecal samples were collected directly from the lubricated rectum with a fecal loop to obtain a minimum of 2 grams (g) of feces, mixed with an equal volume of 10% buffered formalin and placed in sterile containers labeled with identification data. In those cases in which the sample was insufficient, the dog was not included in the study. Samples were kept at 4º C (MABE 13 feet, MABE, Mexico) until examination in the laboratory. Feces were collected without pain or distress under qualified veterinary supervision following appropriate ethical standards.

Parasitological procedures

All the samples were observed macroscopically for visible parasites, color, and consistency and presence of blood or mucus. Each fecal sample of 2–5 g of stool was mixed with 10 mililiters (mL) of saturated sucrose solution (specific gravity: 1.28) in a cup. Mixture was then filtered using a 5 –x -5 centimeters (cm) gauze square [38]. The strained solution was poured into a 15 mL centrifuge tube, filled with sugar solution about 2 cm from the top of the tube and centrifuged (BD Clay Adams, Dynac, Becton

Dickinson, USA) at 500 G for 5 minutes (min). The test tube was then removed and filled to the top with sugar solution. A coverslip was placed in the surface for 10 min, placed in a glass slide and visualized under microscope (model CME, Leica, Germany) to identify helminth eggs. The eggs were differentiated according to their morphologic characteristics [39]. A puppy was classified as positive if at least one egg was present in the fecal sample.

Questionnaire

Before fecal examination, one of the researchers asked for owner's consent in order to obtain the fecal sample and to be interviewed by a face-to-face questionnaire,. The design of survey was based on those previously validated and published [1, 28, 29]. Participation was voluntary. Questionnaire consisted of 15 questions arranged in three sections: 1) related to puppies: management practices (outdoor, or restricted at home -in hard flooring, earthen floor or both-, stray dog), deworming and previous diagnostic procedures; 2) related to owner as the caretaker: frequency of feces collection at home; collection of dog feces throughout walks (yes/no); if not, reason (repulsion, fear, fertilizer, abandoned areas, not important); elimination of feces at home (as household waste; in toilet bowl, in environment), use of personal protection or sanitary measures to collect feces (gloves, hand washing); 3) personal questions (age, instruction level, gender).

Statistical analysis

The prevalence was calculated for each parasite. Statistical differences were set at $P < 0.05$ by a *Chi*-square test. Associations between parasitism and host and management factors were quantified by univariate analyses and odds ratios. In all the cases, statistical program SPSS Statistics 22.0 (IBM, USA) was used [28].

RESULTS AND DISCUSSION

Parasitological results

Helminths eggs were found in 180 of 272 samples (66.3 %). The overall prevalence of zoonotic helminth infections found in the present study is worrying if compared with 35% of prevalence in canines of Zulia State at Western Venezuela [33] or with those reported worldwide, as 52.4% in Argentina [14]; 51% in Perth, Australia [3]; 22.8% in Canada on the island of Saint Pierre [4]; 18.22% in Zambia [5], 7% in Portugal [28] and 6.7% in Iran [16]. There is a report from Ghana with 86.7% [1].

The predominant specie was *Toxocara canis* (45.2%) (TABLE I). Prevalence values of *T. canis* reported worldwide ranged from 5.5 to 64.7% [27] and is the most prevalent parasite in dogs younger than six months of age [14, 23, 28]. Infection en humans occurs when accidentally ingest the microscopic, oval and thickshelled-embryonated eggs (shed in dog feces) containing *Toxocara* larvae by hand-to-mouth contact. Children are particularly prone to infection because they are exposed to the eggs on sandboxes and playgrounds contaminated with dog feces. An early and unique report from Caracas confirmed that 66.6% of children (2-7 years old) suffered toxocariasis [30].

Dipyllidium caninum and *Ancylostoma* spp. were found in 11.8 and 5.5% of the samples, respectively, as single infections (TABLE I). Mixed infections by two species (*T. canis / D. caninum* or *T. canis / Ancylostoma* spp.) were identified in 3% (8/272) of the samples, but none of them presented more than two species. Prevalence values of *D. caninum* and *Ancylostoma* spp. were lower than those of *T. canis* (TABLE I). Both species are more frequent in canines older than six months, unlike *T. canis,* and with higher values of prevalence in dogs from shelters compared with veterinary hospitals or pet stores [2, 3, 13]. No other helminth specie was observed. Many biological factors inherent to the parasite's life cycles successfully facilitate the perpetuation of these parasites. These include vertical transmission, ensuring the early onset of egg production in young definitive hosts, the fecundity of the adult females and the prolonged survival of eggs, e.g. *T. canis* [35], the short period to become an infective stage in tropical climates (e.g. *A. caninum* larvae) [21] and the permanence of fleas (as intermediate hosts of *D. caninum*) in-house microhabitats [37]. The inability to obtain or afford treatments contributes to a higher level of infection because helminthiases in canines are easy to control with adequate therapy [10]. Regis et al. [34] demonstrated in Brazil that dogs whose owners had low incomes were more infected than those attending more expensive veterinary clinics, because in Brazil there are no public veterinary services.

Coccidia oocysts were identified as a single infection in nine (3.3%) of the samples or in two (0.8%) in a co-infection with helminths. The nine dogs with a single coccidian infection were discarded in the data analysis. A 30.5% (83/272) of the samples resulted negative in the microscopic analysis (TABLE I).

TABLE I

PREVALENCE OF INTESTINAL HELMINTHS IN PUPPIES ATTENDING FOR GOVERNMENTAL VETERINARY SERVICES IN CARACAS, VENEZUELA

Diagnosis	*n*	**Prevalence (%)**
Toxocara canis	123	45.2
Dipylidium caninum	32	11.8
Ancylostoma spp.	15	5.5
T.canis / D. caninum	4	1.5
T.canis / Ancylostoma spp.	4	1.5
D. caninum / coccidia	1	0.4
Ancylostoma spp. / coccidia	1	0.4
Infected by helminths	180	66.3
Single infection by coccidia	9	3.3
NOva†	83	30.5
Total	**272**	**100**

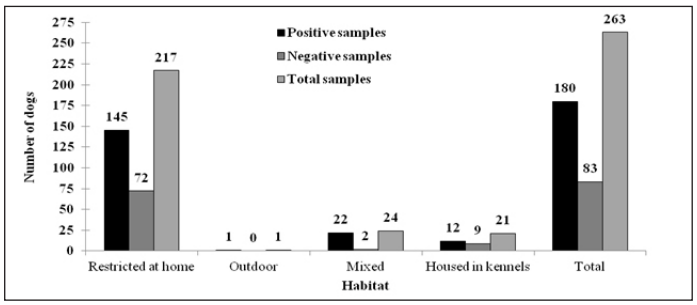
† NOva: No ova was observed in the analysis

There were no significant differences (P > 0.05) related to sex, age or breed of the infected puppies. Most of the puppies (180/272) belonged to hairy breeds (data no shown). Roddie et al. [36] showed that 100% of the parasitized puppies had a total of 30,834 viable *T. canis* eggs in their hair, both on the back and around the anus. As the presence of *T. canis* infective eggs in the hair of dogs is barely considered by veterinarians, it should be considered that infected dogs of hairy breeds may be more likely to harbor parasite eggs in their hair and thus, owners and their children should be aware.

Habitat of the dogs

Concerning outdoor access and positivity to enteric helminths, of 82.5% (217/263) of the puppies exclusively restricted at home, 66.82% (145/217) were parasitized. A 9.12 % (24/263) of the home dogs had also regular outdoor access and 91.66% (22/24) of these puppies were positive to one or two helminths species. A 57.14% (12/21) of samples from "Misión Nevado" puppies had helminth eggs in feces (FIG.1a). The stray dog (1/263) cared by the community was also parasitized. Martínez-Moreno et al. [25] reported higher levels of helminth infections in stray animals.

Of all the puppies restricted at home with a positive diagnosis, five stayed on earthen floor, 124 on hard flooring and 39 had access to both earthen and hard flooring throughout the day (FIG. 1b). There were no statistical differences (P > 0.05) between positive samples and dog habitat.



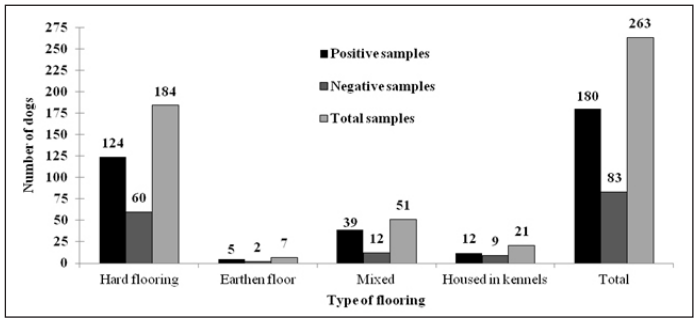


FIGURE 1. RESULTS OF ANALYSIS OF FECES SAMPLES ACCORDING TO a) DOG HABITAT; b) TYPE OF FLOORING IN DOG HABITAT.

Nine samples with a single coccidian infection are not included in the figure.

Risk factors

Each owner reported to be the only caretaker concerning to cleaning and disposal of feces of the dog. Puppies of "Misión Nevado" (*n* = 24) were not considered in the analysis of risk factors. There were no statistical differences between the age or instruction grade of owners (P > 0.05).

In this study, habitat of the dog (restricted, outdoor, both); coproparasitic diagnosis, sex of puppies and instruction level of the owners were not statistical different variables. Gender of the owners, frequency of feces collection at home and unknowledge about zoonoses caused by dog parasites were variables associated to risk factors by univariate analysis (TABLE II).

TABLE II
UNIVARIATE ANALYSIS OF THE VARIABLES ASSOCIATED TO RISK FACTORS AND POSITIVITY TO HELMINTHS

Variable	Category	*Chi*[2]	*P*[*]	Odds ratio[**]
Sex of puppies	Female Male	0.279	0.597	-----
Puppies habitat	Restricted at home Outdoor Mixed Kennels	6.455	0.535	-----
Positive parasitological diagnosis	Yes No	0.433	0.510	-----
Gender of owners	Female Male	4.276	0.039	1.78 (1.02-3.08)[1]
Instruction level of owners	Basic High school University Postgraduate	3.773	0.287	-----
Cleaning	Monthly Weekly Day in between Daily Several times a day Monthly	12.106	0.033	8.42 (1.97-36.11)[1]
Knowledge about zoonoses	Yes No	4.806	0.028	†

[*] Statistical differences set at P < 0.05; ** Confidence level: 95% †Only two people showed knowledge about zoonoses. [1]Risk factor

The number of owners of female gender was 142; 106 were men. The frequency of female owners with positive puppies (142:107) was higher than male owners (106:67) (P < 0.05). Significant differences and a positive association were found

between the gender of the owner and the positive diagnosis to helminthiasis in the pups (TABLE II). Pereira et al. [29] did not find a relationship between the gender of the owners and the positivity to parasitosis, but it was found that women were 1.78 (1.02-3.08) times more likely to have positive puppies than men in the present study. Herzog [18] reported that women, on average, have more positive attitudes toward animals (on their use, protection) while men have high levels of negative attitudes (hunting, animal abuse, less favorable attitudes towards animal protection). Therefore, in this study, women were at high risk of being infected by one or several of the three parasites. Gender roles influence patterns of exposure to infectious agents, for example, where men and women spend their time, and the pathological agents they come into contact with, as well as the nature of exposure, its frequency and its intensity [42]. Women spend more time inside home to care children and animals and to accomplish household tasks; therefore, females tend to face greater exposure to parasites inside the home.

Owners were also asked about the practice of feces disposal, fecal collection through walks and knowledge of zoonoses. Most of the owners (n = 120) reported to clean droppings in a daily basis; 61, a day in between; 15, weekly and one, monthly; 19 owners never collected the feces of their dogs. Among the group of puppies with a daily collection scheme, 64.16% (n = 77) were positive to helminths and of these, 43% (n = 51) to *T. canis* (in a single or mixed infection). In the case of owners who never collected/cleaned droppings or did it in a monthly or weekly basis, almost 100% of their puppies were parasitized (19/17; 1/1 and 15/15, respectively) and most of them with *T. canis* (FIG. 2). Regarding to *D. caninum* or *Ancylostoma* spp. - positive animals, two of their owners never collected/cleaned the feces and three did it in a weekly basis (FIG. 2). Significant differences were found between the collection scheme and positive parasitological diagnosis (P < 0.05). Owners reported to dispose the feces into the bathroom bins, domestic garbage or garbage chute.

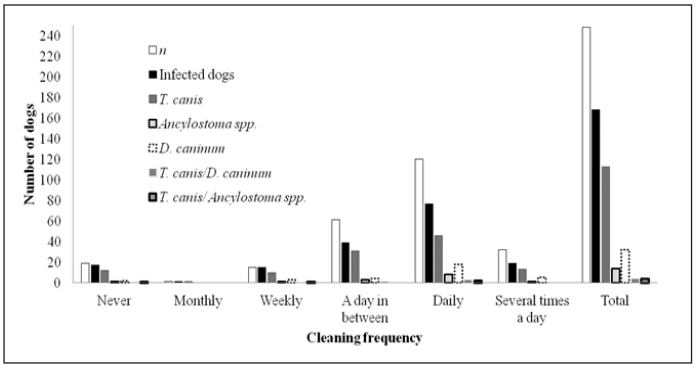


FIGURE 2. FREQUENCY OF CLEANING/RECOLLECTION OF DOG FECES AT HOME

To calculate the odds ratio (OR), the "never, monthly or weekly" collection frequencies were categorized as “unacceptable behavior", because these frequencies allowed the development of the infective forms of the parasites. “Good behavior" included the daily, a day (d) in between or several times a d collection frequencies, because they prevented the maintenance of positive feces at home, the development of the environmental cycle of the parasites and hence, the infection of the dog caretakers or their families. Owners/caretakers who practiced the “unacceptable" frequencies presented 8.42 (1.97-36.11) times more risk of acquiring one of the parasites (TABLE II). In a seroprevalence study of children toxocariasis carried out in Maracay, Venezuela [24], 50-60% of the children had contact or played with the dogs present in parks or near their schools, but there was no relationship between the keeping of dogs at home and seropositivity, so it is important to consider dog caretakers as the most prone group in acquiring the infection because of handling of feces. Congestion of buildings and houses in the center of the cities also contributes to both canine and human infections because there is less free space and a greater concentration of feces [27]. The risk is greater because *T. canis* eggs may be viable for many years [43] and *Ancylostoma* spp. eggs are infective just two-eight days after deposition in environment.

Owners were also asked about collection of their dog´s droppings in their walks (yes/no, if not, reasons): 72% (179/248) did not collect the droppings and left them throughout the walk. The reasons were uneven; 39.7% (98/248) gave no importance to collection; 14.3% (35/248) considered that feces were fertilizers, 11.4% (28/248) of the puppies dropped in abandoned areas and 6.6 % (16/248) reported repulsion to collect the droppings. However, citizens of Municipality are bound by law in the Article 21 of the Reform of the Ordinance on Tenure, Control, Registration, Marketing and Protection of Domestic Animals [7] to collect and remove the feces produced by their animals from the public sites and deposit them in suitable places. A study carried out at the University of Lisboa, Portugal, indicated that 37% of owners do not collect feces in public places [26]. In both cases, the percentage may be higher because respondents could feel judged on the question. If feces are not collected in public places, the possibility of human infection is much greater [22].

A 99.2% (246/248) of the owners admitted ignorance about the term zoonoses or about the fact that dog parasites may be responsible of human diseases. The OR (P 0.028) could not be calculated just because of the low number of positive answers; however, this variable is associated to a risk factor because 246 owners are not aware that family dog may carry zoonotic parasites. Neves et al. [28] found by means of a written questionnaire to 368 dog and cat owners, that 56.5 % of the responders had heard of the word “zoonosis” and 35.2 % of them were aware of the possible transmission of parasites from their pets to themselves, which was a higher level of knowledge about zoonosis than the results of the present study.

Finally, the results indicated that citizens need more and clear information about zoonotic potential of parasites and the veterinarians should have a more proactive role in warning about control schemes to avoid the transmission of parasites to owners.

CONCLUSIONS AND IMPLICATIONS

The study shows that more than 60% of the dogs were infected with helminths. The top parasite was *Toxocara canis*, considered of great public health significance because it is the ethiological agent of the most widespread and economically important parasitic zoonoses. Gender of owners, frequency of feces collection at home and unknowledge about zoonoses caused by dog parasites constituted variables associated to risk factors; women owners are at greater risk of acquiring parasitic zoonoses compared to male owners. Only two owners had knowledge about transmission of zoonotic parasites to humans, what urgently requires an education campaign set up by both governmental health authorities and veterinarians from the public and private sectors. Considering the high percentage of parasitized puppies and the fact that most of the owners ignore the risks of these parasites, not only dogs are in threat of acquiring a parasitic infection, but are a major source to human infections in the Metropolitan District of Caracas.

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