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Endoparasitic infections in dogs from rural areas in the Lobos District, Buenos Aires province, Argentina

Infecções endoparasitas em cães de áreas rurais do distrito de Lobos, província de Buenos Aires, Argentina

Marcela Cecilia Dopchiz[1,2]*; Carla Mariela Lavallén[1,2]; Roberto Bongiovanni[3]; Patricia Verónica Gonzalez[1]; Celina Elissondo[1,2]; Francisco Yannarella[4]; Guillermo Denegri[1,2]

[1]Laboratorio de Zoonosis Parasitarias, Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Buenos Aires, Argentina

[2]Consejo Nacional de Investigaciones Científicas y Técnicas – CONICET, Argentina

[3]Departamento de Bromatología y Zoonosis, Municipalidad de Lobos, Lobos, Buenos Aires, Argentina

[4] Cátedra de Parasitología y Enfermedades Parasitarias, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina

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Abstract

Dogs are definite hosts for several zoonotic helminthes and protozoan. Rural areas from the Lobos District in the northeast of Buenos Aires province, Argentina, are mainly used for livestock activity, increasing in this way the number of dogs on farms as well as the human risk of parasitic infections. The aims of this research were to evaluate the endoparasitic infections in dogs from farms in the Lobos District and analyze their zoonotic importance as well as several risk practices and habits of the rural population. Forty-two dog fecal samples obtained in 21 farms were analyzed through coproparasitological methods and coproantigen tests, which resulted in an overall parasite prevalence of 69.05% and 80.95% of the parasitized farms. The most frequent parasites were *Trichuris vulpis* and *Eucoleus aerophila* (26.19%), *Echinococcus granulosus* (19.05%), *Uncinaria stenocephala* and coccids (14.29%). The analysis of epidemiological files showed several habits of the rural population considered as risk factors associated with the presence of fecal samples parasitized and the presence of *E. granulosus* on the farms. It is clear that people involved with the farms studied were exposed to several helminthes that could cause serious diseases like cystic echinococcosis, which can become an important public health issue and affect the economy worldwide.

Keywords: Parasites, zoonoses, rural areas, dogs.

Resumo

Os cães são hospedeiros definitivos de vários helmintos e protozoários zoonóticos. As áreas rurais do distrito de Lobos, no nordeste da província de Buenos Aires, Argentina, destinam-se principalmente à atividade pecuária aumentando desta forma o número de cães em fazendas, bem como o risco humano de infecções parasitárias. Os objetivos desta pesquisa foram avaliar as infecções endoparasitas em cães de fazendas do distrito de Lobos e analisar a sua importância zoonótica, bem como as práticas de risco e hábitos da população rural. Quarenta e duas amostras de fezes de cães obtidos em 21 fazendas foram analisadas pelos métodos coproparasitológicos e testes de coproantígenos, resultando numa prevalência de parasitas de 69,05% e 80,95% das propriedades parasitados. Os parasitas mais frequentes foram *Trichuris vulpis* e *Eucoleus aerophila* (26,19%), *Echinococcus granulosus* (19,05%), *Uncinaria stenocephala* e coccídeos (14,29%). A análise dos arquivos epidemiológicos mostraram vários hábitos da população rural como fatores de risco associados com a presença de amostras fecais parasitadas e a presença de *E. granulosus* em propriedades rurais. É evidente que as pessoas das fazendas estudadas foram expostas a vários helmintos que podem causar doenças graves como equinococose cística, que constitui importante problema de saúde pública e econômico a nível mundial.

Palavras-chave: Parasitas, zoonoses, áreas rurais, cães.

*__Corresponding author:__ Marcela Cecilia Dopchiz
Funes 3350, Mar del Plata, 7600, Argentina
e-mail: mdopchiz@mdp.edu.ar

Introduction

Several studies performed throughout the world have demonstrated that dogs can play an important role in the transmission of over 60 zoonotic infections (MacPHERSON, 2005). Dogs are definite hosts for several zoonotic helminthes and protozoan like *Echinococcus granulosus*, *Ancylostoma* spp., *Toxocara canis*, *Trichuris vulpis*, coccids, among others, which are pathogenic in humans (SORIANO et al., 2010). Since the number of dogs bred in rural areas and in cities has recently been increasing worldwide, the human risk of parasitic infections has been expected to increase as well (DUBNÁ et al., 2007). Different cases of human infections caused by *Trichuris* spp., *Hymenolepis nana*, *E. granulosus*, *Giardia lamblia* and *Toxocara canis*, were reported in the provinces of Buenos Aires, Neuquén, and Salta, in Argentina (TARANTO et al., 2000; BASUALDO et al., 2007; CESANI et al., 2007; PIERANGELI et al., 2007; DOPCHIZ et al., 2011; GAMBOA et al., 2011). One important parasitic disease is the cystic echinococcosis (CE), an infection of humans and herbivorous animals, caused by the larval stage of the parasite *E. granulosus*. In the province of Buenos Aires, CE is considered an important public health and economic problem for both human beings (DOPCHIZ et al., 2009, 2011) and livestock (DOPCHIZ, 2006). Since 2007, some producers from the Lobos district requested veterinary assistance regarding CE and there was not any background about the epidemiology, the prevalence and the incidence of the infection in all the hosts, as well as the CE infection risk for humans. There was not information about other dog parasitic diseases in the region either. Therefore, this work aimed to evaluate the endoparasitic infections in dogs from farms in the Lobos district and to analyze their zoonotic importance, as well as several risky practices and habits of the rural population.

Materials and Methods

1. Study area

The study area is the Lobos District, situated in the province of Buenos Aires (35° 10' S and 59° 05' W). It is located in the north of the Río Salado, with an area of 1,740 km^2. According to the last census in 2010, the total population was about 36,172 inhabitants with a density of 20.78 inhab/km^2 (INDEC, 2010). Between 55 and 60% of its productive area is used for livestock activity, 35-40% for agriculture, while the remaining 5% is used for raising pigs and sheep, as well as for beekeeping and horticulture.

2. Source of samples and epidemiological files

To perform a representative sampling of the study area universe, 21 farms were selected by systematic random using the list of producers given by the SENASA (Servicio Nacional de Sanidad y Calidad Agroalimentaria).

Forty-two samples of fresh dog feces were collected from all the farms and conserved in plastic containers in duplicate. Group A was kept with 10% formaldehyde to perform the coprological analysis and group B was frozen until its utilization in the coproantigen tests.

An epidemiological file was kept on each farm. If the farm had more than one post, the questionnaire was performed to only one of them. It began with questions that included general information about the farm and the practice of home slaughtering. Then, questions about the number of dogs, their feeding habits and deworming, and also questions about culture, hygiene and consumption of raw vegetables were asked. Finally, the questionnaire concluded with some questions to associate risk factors with dog echinococcosis and human cystic echinococcosis. Questions were formulated in order to have clear and short answers and to avoid misunderstandings in translation.

3. Coprological exams

Group A samples were processed in the parasitological laboratory of the Municipal Center of Zoonoses (MCZ) of General Pueyrredón according to both the Ritchie's sedimentation and Sheather's flotation methods (MÉNDEZ, 1998). Two slides were prepared and microscopically examined at 100×, 400× and 1000× magnifications for each sample. Identification of parasites was performed through morphological and morphometric characteristics. A sample was recorded as positive if at least one parasitic form was observed by any of the methods used.

4. Coproantigen procedure

Coproantigen determination was performed through the Copro-ELISA as screening test and the Copro-Western blot method as a confirmatory test to demonstrate *E. granulosus* antigens in dog feces collected on the farms. In carriers dogs of *E. granulosus* the sensitivity and specificity of the complex copro-ELISA + copro-Western blot is 100%, while the prevalence found by this system is superior than the one obtained by the arecoline test (45.4% and 33.3% respectively) (GUARNERA et al., 2000).

In this way, group B of samples was inactivated at –18 °C and then sent to the Parasite Immunology Laboratory, Parasitology Department, INEI ANLIS "Carlos Malbrán" (Buenos Aires, Argentina), following the general indications to transport biological material. The Copro-ELISA test was performed following the technique described by Baronet et al. (1994) and modified by Guarnera et al. (2000). All the positive samples determined were processed through the confirmation test Copro–Western Blot to identify *E. granulosus*, following standard techniques (TOWBIN et al., 1979; ALLAN et al., 1992). The Copro-ELISA cut-off values were determined by employing 25 fecal samples from healthy dogs. Mean sample value plus three standard deviations was 0.215 and all examinations surpassing this value were considered positive. The Copro-Western blot was considered positive by the presence of two bands of molecular weights 40 and 45 kDa. This diagnostic system was considered indeterminate when the Copro–ELISA was positive and the Copro–Western Blot was negative and it was considered positive when both techniques were positive (GUARNERA et al., 2000).

5. Data analysis

Data were tabulated and analyzed using the Epi Info™ software (Version 3.3.2, CDC, Atlanta, USA). Odds Ratio (OR) with a confident interval of 95% and *p* probability value were calculated through a simple table analysis using Statcalc, to identify the association between risk factors (like dogs treated with anthelmintic less than 3 times per year, dogs not treated with anthelmintic, dogs fed with raw offal, offal exposed in the field and home slaughter) and the dog zoonoses found.

Results

The analysis of the epidemiological files showed that the mean area of the studied farms was 324.12 ha, ranging from 3 to 1790. All the farms possessed a mean number of 1.1 ± 0.19 houses ranging from 1 to 3. The total number of dogs reported on all the farms was 107, with a mean number of 5.1 ± 3.1 dogs per farm, ranging from 2 to 14.

Parasites were found in 80.95 % of the farms visited; 52.94% and 47.06% of dogs presented single and multiple parasitic infections, respectively. The overall prevalence of parasites was 69.05% among the 42 fecal samples analyzed. The most frequently observed parasites were *T. vulpis* and *Eucoleus aerophila* (26.19%), followed by *E. granulosus* (19.05%), *Uncinaria stenocephala* and coccids (14.29%) and others with minor percentages. General and relative prevalence of each parasite in fecal samples and on the farms are shown in Table 1. The parasites found in the single parasitic infections were *E. aerophila* (28.57%), *E. granulosus*, *A. caninum*, *T. vulpis* (21.43% each one) and *Taenia* spp. (7.14%). In the multiple parasitic infections, the most frequent parasite association was between *E. aerophila* and *E. granulosus* (20%). Fecal samples with taeniid eggs did not coincidence with positive samples for the coproantigen test. In this way was excluded from the group of taeniids.

The epidemiological data analysis showed that dogs were improperly dewormed (inappropriate drug and/or incorrect administration) in 81% of the farms; 76.5% of them presented fecal samples parasitized. In the remaining 19%, dogs were properly dewormed and parasites were found in all of them. In 52.38% of the farms investigated, people consumed raw vegetables after washing them with different techniques. Parasites were found in 90.90% of them. In 42.86% of the farms, people grew their own vegetables. Parasites were found in all of them.

Questionnaires (n = 21) were evaluated for risk factors associated with the presence of fecal samples parasitized on farms as well as the presence of *E. granulosus*. Univariate analysis demonstrated that there was no significant association between dogs treated with anthelmintic more than thrice per year and the presence of fecal samples parasitized. The same analysis was done to evaluate the association between different risk factors and the presence of *E. granulosus*. The OR obtained had a very ample CI and showed that there was no statistically significant association.

Discussion

To understand the epidemiology of zoonotic parasites it is important to reduce the risk of infections in human beings. Zoonoses involving dog parasites are usual and important at the same time, causing, some of them serious diseases (DUBNÁ et al., 2007). In the present study, the high number of dogs found per farm added to the close contact between them and human beings, specially in this region where livestock raising is economically important, generate potential risks of acquiring zoonotic diseases for the rural populations. This finding corroborates results found by Pierangeli et al. (2007) and Soriano et al. (2010) in the province of Neuquén, Argentina.

Nematodes are involved in several human infections. Trichuroidea such as *T. vulpis* and *E. aerophila* are parasites whose zoonotic potential is frequently disputed. Nevertheless, some researchers reported visceral larva migrans syndrome and enteric trichurosis caused by *T. vulpis* in adults and children (SAKANO et al., 1980; MASUDA et al., 1987), and some cases of human capillariosis caused by *E. aerophila* that may induce relevant damage resembling bronchial carcinoma (LALOŠEVIC et al., 2008). The high prevalence of these parasites found in this study represents an important risk to develop some of these parasitic diseases in the human population. The endoparasite *T. vulpis* was more prevalent than reported by other studies carried out in rural areas from Nigeria (14.2%) and the Czech Republic (1.7%)

Table 1. General and relative prevalence of each parasite in fecal samples from farms in the Lobos district, Buenos Aires - Argentina.

Parasites	Nº of positive samples	General prevalence (n = 42[a]) (%)	Relative prevalence (n = 29[b]) (%)	N° of farms with parasites	General prevalence (n = 21[a]) (%)	Relative prevalence (n = 17[b]) (%)
E. aerophila	11	26.19	37.93	7	33.33	41.18
T. vulpis	11	26.19	37.93	6	28.57	35.29
E. granulosus[c]	8	19,05	27.59	5	23.81	29.41
Coccidios	6	14.29	20.69	3	14.28	17.65
U. stenocephala	6	14.29	20.69	2	9.52	11.76
A. caninum	5	11.90	17.24	5	23.81	29.41
Taenia spp.	4	9.52	13.79	3	14.28	17.65
Total[d]	29	69.05	100	17	80.95	100

[a]General prevalence was estimated in relation to total number of samples analyzed; [b]Relative prevalence was estimated in relation to total number of positive samples; [c]Prevalence of parasites was estimated taking into account positive results in the Copro–ELISA and the Copro–Western Blot tests; [d]More than 1 parasite agent can be present in a single sample; Nº - number, % percentage.

(DUBNÁ et al., 2007; UGBOMOIKO et al., 2008). In Argentina, Soriano et al. (2010) reported a prevalence of 1.3% in rural areas of Neuquén. Both trichuroid are found in wild animals like foxes, wolves and weasels among others (WEESE; FULFORD, 2011; SANTOS et al., 2012). Taking into account that a high density of foxes was reported in Buenos Aires province (between 0.46 and 1.62/foxes/km^2) (PORINI; RAMADORI, 2007) and that they share the habitat with dogs on farms, fox feces could contribute as a source of parasitic infection for dogs.

E. aerophila was previously found in urban dogs from the provinces of Buenos Aires and Neuquén (FONTANARROSA et al., 2006; SORIANO et al., 2010; LAVALLÉN et al., 2011). The present work presented the first report from rural areas and the highest prevalence ever reported in the country. The life cycle of *E. aerophila* could be direct or indirect, with earthworms as intermediate hosts (AFTANDELIANS et al., 1977; RADMAN et al., 1986). It is known that the rural areas of Buenos Aires present soils rich in humus, where the population of earthworms is favored by livestock systems with pasture rotation (SÁNCHEZ; REINES, 2001). The fact that all studied areas were livestock farms could increase the possibility of dogs being in contact with earthworms, which could potentially infect them with this parasite.

In the multiple parasitic infections, the most frequent association was between *E. aerophila* and *E. granulosus.* Since their mode of transmission is different, this association may be due to a higher susceptibility, in certain hosts, to multiple parasitisms. People and animals that live on farms with parasite richness have high possibilities to develop more than one parasitic disease.

Several habits that could be considered risk factors to the infection with zoonotic parasites were observed in the epidemiological files analyzed. Most farms practiced an improperly dog-deworming method, because managers used inadequate drugs or they repeated the treatment less than three times per year. Consequently, parasites were found in a high number of them. However, parasites were also found on all the farms where managers had informed correct dog deworming. One possible explanation is that anthelmintic only kills adult forms, in this way eggs could be found in feces of dogs recently dewormed. Another reason could be that managers answered the questions related to dogs deworming incorrectly. A relevant risk factor is growing vegetables in areas near the places where dogs usually defecate. However, as farmers built up fences around their orchards, the possibility of vegetables being contaminated with parasites might be decreased.

There is a lot of literature about the techniques for the detection of canine echinococcosis, including the coproantigen test. The simplicity and economy of sample collection and preservation systems, the possibility to obtain specimens in geographical areas of difficult access, and the acceptable sensitivity and specificity of the method jointly grant to copro-ELISA + copro-Western blot a high epidemiological value as a surveillance system of cystic echinococcosis (CAVAGIÓN et al., 2005). The usefulness of this test has been validated in Argentina as part of a monitoring system for existing control programs (BARNES et al., 2012). It has also been recommended by the OPS and the OMS (ARGENTINA, 2009), who suggest using the copro-PCR technique only when the coproantigen tests show negative results, because of the high sensitivity and specificity of this method.

The prevalence of farms infected with *E. granulosus* found in this study (23.80%) was higher than those reported by Dopchiz (2006), who used the same techniques, on farms from General Pueyrredon District, located in the southeast region of Buenos Aires province, an area historically hyperindemic for echinococcosis. On farms from the Argentinian Patagonia, Cavagion et al. (2005) found prevalence between 2.9% and 13.9% in provinces undergoing control programs, and 6.3% in a province with no control program, using the same techniques. These results strongly suggest that, although the study area was always considered of low endemicity for being located in the north of the Río Salado, the lack of strategies for disease control has allowed the increase and dispersion of echinococcosis in that region.

Despite the same morphology makes all taeniid eggs indistinguishable, in this work *E. granulosus* was excluded from the group of teaniids because it was identified by the coproantigen technique while the taeniid eggs were recovered by the coproparasitological techniques, and there was no coincidence between these samples.

The answers obtained from the epidemiological files showed situations or practices that favor the transmission of echinococcosis in the region, such as: a) home slaughter, b) offal exposed in the field without any treatment like incineration or burial, c) dogs fed with raw offal, and d) dogs treated improperly with anthelmintic (inappropriate drugs or wrong periods of administration). These factors were worldwide considered the riskiest (BUISHI et al., 2005; MORO et al., 2005; MORO; SCHANTZ, 2009; ROJO-VAZQUEZ et al., 2011), so they were used to perform the univariate analysis. The OR obtained showed that there was no statistically significant association between risk factors and the presence of specific antigens of *E. granulosus* in dogs fecal samples from the farms studied. The wrong anthelmintic treatment and the lack of it were independent from the presence of antigens. The habit of feeding dogs with raw offal and the inadequate offal disposition in the field did not affect the presence of antigens in the fecal samples of dogs. Despite the high frequency of farms that cultivated those habits, the lack of significant association with *E. granulosus* transmission could be explained by the small sample size under study and because the survey was global for the whole farm, so not all the farm posts were considered. Because these posts had different management of offal and sanitary and feeding of dogs, it would be necessary to develop a study in which every farm would be taken as a unit, filling a file in each farm post and considering epidemiological conditions that were not evaluated in this study. Although risk factors associated with CE did not affect the presence of *E. granulosus* in the samples analyzed, the practices reported constitute a risk for CE, so they should be avoided to prevent the transmission of this parasite.

In conclusion these environments may constitute sites of risk for human and animal health, since several risk factors associated with the infection and transmission of zoonotic parasites were observed, and a high prevalence of infected fecal samples were found.

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