

ECHINOCOCCOSIS AND OTHER PARASITIC INFECTIONS IN DOMESTIC DOGS FROM URBAN AREAS OF AN ARGENTINEAN PATAGONIAN CITY

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Abstract In urban populations of South America, dogs with free access to public areas represent a public health concern. The primary consequence of roaming dogs on human health is the transmission of infectious and parasitic diseases mainly through feces contamination. The main diseases likely to be transmitted are hydatidosis or echinococcosis, *larva migrans*, and giardiasis. In Argentina, hydatidosis ranks among the most prevalent zoonosis. Although it is considered a rural disease, the circulation of this parasite in urban areas has been documented. The aim of this work was to survey intestinal parasites in canine feces from two low-income urban neighborhoods of Bariloche city, Argentina, and to assess their seasonal variation. During 2016, 188 fresh dog feces were collected from sidewalks in 40 randomly selected blocks from the neighborhoods. Each sample was processed by Sheater flotation and tested for a coproantigen (CAg) by ELISA. The percentage of parasitized feces was 65.3% (95% CI: 55.9%-73.8%). Eleven parasite species were found, 3 protozoan, 3 cestodes, and 5 nematodes. *Echinococcus* sp. was present in 9.3% of the samples (95% CI: 4.7%-16.1%). Canine echinococcosis rates resulted similar to rates found previously in other neighborhoods of the city. The life cycle of *Echinococcus* sp. is sustained in urban areas by the entry of parasitized livestock, domiciliary slaughtering, and inadequate deposition of offal. The risk of *Echinococcus* sp. transmission to people in these neighborhoods is very high, due to high density of free-roaming dogs and high percentages of infected feces, similar to percentages observed in rural areas.

Key words: zoonosis, stray dogs, dog feces, *Echinococcus* sp., low-income populations

Resumen *Echinococcosis y otros parásitos que infectan a perros domésticos en áreas urbanas de una ciudad de la Patagonia argentina.* En las poblaciones urbanas de América del Sur, los perros con acceso libre a áreas públicas representan un problema de salud pública. La principal consecuencia es la transmisión de enfermedades infecciosas y parasitarias a través de la contaminación por heces. Las principales enfermedades que pueden transmitirse son hidatidosis, *larva migrans* y giardiasis. En Argentina, la hidatidosis es una de las zoonosis más prevalentes y aunque es considerada una enfermedad rural, algunos estudios muestran la circulación de este parásito en zonas urbanas. El objetivo fue registrar los parásitos intestinales en heces caninas de dos barrios de bajos ingresos de la ciudad de Bariloche, Argentina, y evaluar su variación estacional. Durante 2016, se recolectaron 188 heces frescas de perros en 40 manzanas seleccionados aleatoriamente. Las heces se procesaron mediante flotación de Sheater y una prueba ELISA de coproantígeno (CAg). El porcentaje de heces parasitadas fue del 65.3% (IC 95%: 55.9%-73.8%). Se encontraron 11 especies de parásitos, 3 protozoos, 3 cestodos y 5 nematodos. *Echinococcus* sp. estuvo presente en el 9.3% de las heces (IC 95%: 4.7% -16.1%). La equinococosis canina mostró valores similares a estudios previos en otros barrios de la ciudad. El ciclo de vida *Echinococcus* sp. se mantiene en las zonas urbanas por entrada de ganado parasitado, faena domiciliaria y deposición inadecuada de vísceras. El riesgo de transmisión de *Echinococcus* sp. en estos barrios es alto, debido a la alta densidad de perros sueltos y al alto porcentaje de heces infectadas, similar al de las zonas rurales.

Palabras clave: zoonosis, perros sueltos, heces caninas, *Echinococcus* sp., poblaciones de bajos ingresos

In urban and suburban populations of South America, companion animals and dogs with free access to public areas pose a public health risk. The main consequences of roaming dogs on human health are the transmission of infectious and parasitic diseases, the generation of unhealthy foci due to open trash bags, bites, and traffic accidents¹.

Parasitic diseases are an important cause of morbidity. Poverty, poor personal hygiene, lack of sanitation and potable water supply, productive activity and habits of the community, along with feeding and defecation practices of pets play a significant role in the transmission of zoonotic diseases². The main diseases likely to be transmitted by dogs are hydatidosis, *larva migrans* (toxocariasis and ancylostomiasis), and giardiasis²⁻⁵. In Patagonian cities, the zoonotic protozoa and helminthes recorded to date in canine feces are: *Sarcocystis* spp., *Entamoeba* spp., *Giardia* spp., *Isoospora* spp., *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis*, *Capillaria* spp., *Uncinaria* sp., *Ancylostoma caninum*, *Strongyloides* sp., *Dypillidium caninum*, *Taenia* spp., *Diphyllobothrium* sp., and *Echinococcus* sp.⁶⁻¹⁰.

Hydatidosis is a zoonotic disease caused by species of the genus *Echinococcus*. The human infection occurs after the ingestion of *Echinococcus* eggs through contaminated foods or by direct contact with parasitized dogs. Hydatidosis produces morbidity, disability and death, affecting the health of the population and the regional economy^{3,11}. Echinococcosis is a cosmopolitan zoonotic disease and one of the most prevalent zoonosis in rural areas from southern Brazil, Chile, Peru, Uruguay, and Argentina³. Risk and prevention factors have been extensively studied in rural areas of these countries. The main risk factor is age of children, while drinking water at home appears as a protection factor³. The most effective and efficient way to control hydatidosis is primary prevention. Thus, it is indispensable to develop health education to motivate the population to cooperate in avoiding infection of children, dogs, and peridomicile³. Although hydatidosis is considered a rural disease¹², some studies show the circulation of the parasite in urban areas¹⁰. In Europe, *Echinococcus multilocularis* reached urban areas with the invasion of foxes and dogs preying on infected rodents¹². In developing countries, the habit of slaughtering livestock at home in urban areas, and feeding dogs with infected viscera, could favor the translocation of *E. granulosus* to cities¹³⁻¹⁶. Caldas et al.¹¹ reported that between 5.7% and 19.3% of dogs from different rural areas in Argentina were infected with *E. granulosus*. In this country, many deaths occur each year from hydatidosis, with a death rate of 2.7% between 2009 and 2013¹¹. Studies in Patagonia found that between 1.2% and 11.0% of dog feces in urban areas are infected with echinococcosis^{10,17}.

In the Province of Río Negro, Argentina, the hydatidosis control program was launched in 1980 primarily

aimed to deworm dogs. In 1986, ultrasound surveys of children showed a prevalence of 5.6%, and the percentage dropped to 0.3% after a decade of government control programs^{13, 18, 19}.

Low income neighborhoods of Bariloche city, Río Negro, are characterized by rural migration and flow between the countryside and the city. People in these neighborhoods preserve many rural practices such as domiciliary slaughter of sheep and goats²⁰. A parasitological study of dog feces in a low-income neighborhood of Bariloche found that 11.0% were infected with *E. granulosus*¹⁰. A demographic survey of canine population performed in two low income neighborhoods showed that 87% of the houses have at least one dog, with a household/dog ratio of 2.2, and 56% of owned dogs are allowed to roam in the streets²¹. Therefore, it is an ideal scenario for transmission of zoonotic parasites. The objective of this study is to continue a survey of intestinal parasites in dog feces in neighborhoods from Bariloche. In this opportunity, we focus on two low-income neighborhoods assessing seasonal changes in parasite prevalence. Special emphasis is put on the presence of echinococcosis for its consequences on public health, including the discussion of risk factors for humans and measures to avoid human infection.

Materials and methods

The selected neighborhoods are located in the southern side of Bariloche city (41° 10'S - 71° 18'W) (Fig. 1). They comprise an area of 85 blocks (100 m x 100 m each). In 2010 the population of the study area was of 5877 people²². These are fast growing neighborhoods with an estimated 20% population increase in the last 6 years. We assume that about 7100 people inhabit the area nowadays. According to the 2010 census²² between 15% and 57% of the households had at least one unsatisfied basic need.

Forty blocks in the study area were randomly selected. Canine feces were collected in summer, fall, and winter, 2016. One fresh feces sample was gathered from the sidewalk of each block in each of the three seasons. A total of 118 samples were obtained: summer (N = 38), fall (N = 40), and winter (N = 40).

Each feces sample was collected in an individual bag and kept frozen until processed. The Sheeter flotation method was used. Two slides of each sample were microscopically examined at 100X and 400X amplifications. Identification of eggs, cysts, and larvae of parasites was performed by morphological characteristics²³.

In order to determine the presence of *Echinococcus* sp., each fecal sample was mixed in equal parts of 0.15 M PBS with 0.3% Tween 20, vortexed vigorously, and centrifuged at 3500 rpm for 30 minutes at room temperature. The supernatant was removed and stored at -20 °C until further processed. The Coproantigen (CAg) ELISA test was performed according to Pierangeli et al.²⁴. All fecal samples and controls were analyzed in duplicate. Samples with an optical density (OD) value above or equal to the optimal cut off value (OD 0.235) were classified as positive.

Exact binomial 95% confidence intervals (95% CI) were calculated for the overall percentage of infected feces and by parasite species. Prevalence of parasitized feces between

studied periods were compared using the Exact Fisher test. All analyses were performed with the R 3.2.2 package²⁵.

Results

Eleven parasite species were recorded: 3 protozoan, 3 cestodes, and 5 nematodes. A total of 77 parasitized feces were found (65.3%, 95% CI = 55.9%-73.8%). Single infections were present in 42 feces (35.6%, 95%CI:

27.0%-44.9%) while multiple infections were observed in 35 feces (29.7%, 95% CI: 21.6%-38.8%). For each parasite species, the percentage and 95% confidence interval of infected feces overall and by month of survey, are presented in Table 1. The most frequent parasite was *Trichuris* sp. (39%) followed by *Diphylobothrium* sp. (17%). The percentage of feces infected with *Echinococcus* sp. was 9.3%.

The overall percentage of infected samples in summer was 60.5% (95% CI: 43.4%-76.0%), fall 70.0% (95% CI: 53.5%-83.4%) and winter 65.0% (CI = 48.3%-79.4%). The variation was not statistically significant (p = 0.6963). The *per* species analysis of season variation was not statistically significant for any of them (p-value > 0.1 for all species). *Giardia* sp. was found only during the summer, while *Dipylidium caninum* was found only in the fall. It is worth noting that the most zoonotic species (*Amoeba* sp. cysts, *Echinococcus* sp., and *Toxocara* sp.) were found in the three studied seasons.



Fig. 1.– Geographic location of Bariloche city

Discussion

Studies performed in cities from Southern Argentina and Southern Chile found that the average numbers of dogs *per* household range between 0.6 and 1.4, rising up to 2.4 in some neighborhoods²⁶⁻²⁸. Although this is a problem *per se*, the main problem is related to the characteristics of pet ownership. The percentage of owned dogs allowed to roam freely found in these studies varies between 22% and 51%²⁶⁻²⁸. There are no estimates of demographic parameters of the dog population in Bariloche. However, visual inspection and surveys performed in some neighborhoods give a clear indication of a street dog problem²¹.

TABLE 1.– Percentage of infected canine feces by parasite species in 118 samples, by season of collection, and 95% confidence intervals in two low-income neighborhoods of Bariloche city, Río Negro, Argentina

Parasite	Summer		Fall		Winter		Total	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI
<i>Giardia</i> sp.	2.6	0.1-13.8	0.0	0.0-8.8	0.0	0.0-8.8	0.8	0.0-4.6
<i>Amoeba</i> sp. cyst	7.9	1.7-21.4	2.5	0.1-13.2	5.0	0.6-16.9	5.1	1.9-10.7
<i>Coccidia</i>	10.5	2.9-24.8	7.5	1.6-20.4	0.0	0.0-8.8	5.9	2.4-11.8
<i>Diphylobothrium</i> sp.	10.5	2.9-24.8	17.5	7.3-32.8	22.5	10.8-38.5	16.9	10.7-25.0
<i>Dipylidium</i> sp.	0.0	0.0-9.3	2.5	0.1-13.2	0.0	0.0-8.8	0.8	0.0-4.6
<i>Echinococcus</i> sp.	7.9	1.7-21.4	15.0	5.7-29.8	5.0	0.6-16.9	9.3	4.7-16.1
<i>Capillaria</i> sp.	5.3	0.6-17.7	0.0	0.0-8.8	10.0	2.8-23.7	5.1	1.9-10.7
<i>Strongyloides</i> eggs	2.6	0.1-13.8	5.0	0.6-16.9	0.0	0.0-8.8	2.5	0.5-7.3
<i>Strongyloides</i> larvae	0.0	0.0-9.3	2.5	0.1-13.2	2.5	0.1-13.2	1.7	0.2-6.0
<i>Toxascaris</i> sp.	15.8	6.0-31.3	15.0	5.7-29.8	5.0	0.6-16.9	11.9	6.6-19.1
<i>Toxocara</i> sp.	7.9	1.7-21.4	17.5	7.3-32.8	12.5	4.2-26.8	12.7	7.3-20.1
<i>Trichuris</i> sp.	34.2	19.6-51.4	40.0	24.9-56.7	42.5	27.0-59.1	39.0	30.1-48.4

The richness in parasite species (11) in our study was higher than observed in Mexico²⁹ (6 species) and Peru³⁰ (5 species) but lower than species richness reported in Santiago, Chile where 16 different parasite species were found in dog feces³¹. However, it is worth noting that this latter work analyzed 972 dogs with gastrointestinal symptoms. The richness of parasite species in dog feces from Patagonian cities varies between 10 and 11^{7, 9, 32}. The estimated overall percentage of infected feces in the neighborhoods studied herein was 65.3%, a value which is within the range of other cities in the Patagonian region; for example, the prevalence of infected feces reported in Comodoro Rivadavia, Chubut, was found to range between 46.6% and 86%^{7, 32}. This last value was obtained in a very low-income neighborhood near the urban solid waste disposal facility. The percentages of infected feces were also similar to those found in other Latin American studies like Mexico (73.3%), Peru (40.1%), and Chile (64.8%)²⁹⁻³¹. Previous studies in other neighborhoods of Bariloche found between 37% and 67% of parasitized feces, and the composition of the parasite communities was very similar to the one observed in this study¹⁰.

As in previous works, no clear seasonal infection patterns were observed in our study, as seasonal variations did not reach statistical significance in parasites recovered throughout the study period. Similar results were reported in other countries with temperate climate like the USA and Spain. Seasonal variations were reported for ascarids and *Giardia* sp. with higher infection values in fall and winter^{33, 34}. We found the ascarid *Toxocara* sp. in all three seasons with the highest infection value in fall. In contrast, we only recorded the presence of *Giardia* sp. in summer. Hookworms also show different seasonal infection patterns around the world; e.g. in the USA these species showed maximum infection values in spring and summer³³ while in Spain *A. caninum* showed maximum values in fall and winter³⁴.

The World Health Organization (WHO) suggested that the control of echinococcosis is a priority within the framework of "care for neglected diseases in deferred populations"¹¹. As mentioned, echinococcosis is considered a rural zoonosis; however, numerous cases were found among both urban dogs and people who never left the cities, indicating that the disease is cycling in urban areas of South America^{35, 36}. In Peru, stray dogs infected with *E. granulosus* were found in areas surrounding slaughterhouse, where offal of infected animals is improperly discarded due to non stringent supervision; moreover it is possible that infected offal reaches city markets through informal channels^{36, 37}. The percentage of dog feces infected with *Echinococcus* sp. in our study was 9.3%, similar to values reported in urban areas of Chile (8-15%)¹⁵, but higher compared to Bolivia (3.4%) and Brazil where no CAg was found in feces^{16, 38}. The prevalence of echinococcosis in our study is 10 times

higher than values reported in other cities of the region^{9, 16, 17, 32}, and similar to prevalences in rural areas^{9, 16, 32, 39-40}. A previous study in other low-income neighborhoods of Bariloche found a similar prevalence of echinococcosis in dog feces¹⁰, showing persistent transmission foci in urban neighborhoods with rural habits due to human mobility between the countryside and the city²⁰.

In Argentina, human cases of hydatidosis have been detected in cities¹⁶, particularly in children under 10-year-old, and many of these cases were unrelated to rural areas. This situation is relatively recent, thus *Echinococcus* sp. life cycle is maintained in urban areas subsidized by uncontrolled entry of parasitized livestock, domiciliary slaughtering, inadequate deposition of offal¹⁶, and by the lack of knowledge on the risk involved in these practices, along with inadequate dog care. In the neighborhoods included in this study, people who never left the city were found to harbor cysts (personal observation).

These neighborhoods are characterized by an expansive population pyramid (almost 30% under 12-year-old) and low educational level. These demographic characteristics are factors favoring the risk of transmission of zoonotic diseases, especially hydatidosis³. Many of the preventive measures are difficult to apply. For example, frequent washing of hands and vegetables is not possible in some households because 22.1% lacks home water supply and 11.4% lacks access to safe drinking water⁴¹. Regarding preventive measures related to dog care, almost half of the dog population has free access to the street²¹. Although 83.4% of the dogs were dewormed, they were treated only once during the previous year²¹, while the recommendation is to deworm free roaming dogs at least 4 times a year⁴². Besides, there is no government deworming control program for dogs in the city. Also, sheep slaughter and fish evisceration with inadequate offal and discard disposal are rather common practices in these neighborhoods. In agreement with these observations, we found *Diphyllobothrium* sp. in 16.9% of the feces, an infection acquired by dogs consuming raw viscera of infected fish, and 9.3% of feces were positive for *Echinococcus* sp. Thus, the dispersion of parasite eggs is promoted by large numbers of dogs roaming without control²¹; it should be taken into account that dogs defecate within 200 m of their houses⁴³. Additionally, the climate in Bariloche favors parasite egg survival; indeed, eggs were shown to survive long in the environment at low temperatures (7 °C) and remain viable for up to 294 days³. More recently, some studies have found that eggs can remain infective after 41 months under arid conditions in certain regions of the Argentine Patagonia³.

In conclusion, in this study we found high rates of parasite infected dog feces, including potentially zoonotic species, in two low-income neighborhoods of Bariloche. These findings raise a public health alert. Several factor –namely high numbers of dogs roaming freely in the

streets, lack of systematic canine deworming, domiciliary livestock slaughter and inadequate disposal of offal, among others— provide suitable conditions for the maintenance of parasites in the urban environment. Joint governmental-private initiatives should be promoted in order to address this multifactorial problem in low-income neighborhoods of Bariloche.

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Clinical research is concerned with sick patients and thus with man and his diseases. But like other branches of science it uses the knowledge, techniques, and ideas of kindred disciplines. Many clinical research workers also use animals for experiments and for reasons that are obvious. But it must always be remembered that, so far as human disease is concerned, the first observation and the final proof, in so far as can ever be final, is made in man, there is no substitute. Thus if the relief of human suffering and the improvement of human society are the objects of medical research, observation and experiment in man are the lynchpin of the whole structure. This needs re-emphasis, because it is sometimes forgotten.

La investigación clínica se refiere a enfermos y por lo tanto al hombre y sus enfermedades. Pero, como otras ramas de la ciencia usa el conocimiento, técnicas e ideas de disciplinas relacionadas. Muchos investigadores clínicos también usan animales para sus experimentos, y por razones que son obvias. Pero se debe recordar siempre que, en cuanto a enfermedades humanas se refiere, la primera observación y la prueba final, tanto como alguna vez puede ser final, se hace en el hombre. Por consiguiente si el alivio del sufrimiento humano y la mejora de la sociedad son el objeto de la investigación médica, la observación y el experimento en el hombre son el eje de toda la estructura. Esto necesita re-enfatizarse, porque a veces se olvida.

George W. Pickering (1904-1980)

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