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Presence and persistence of intestinal parasites in canine fecal material collected from the environment in the Province of Chubut, Argentine Patagonia

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Abstract

We investigated the presence of intestinal parasites in canine feces collected from public squares in Comodoro Rivadavia, Chubut, Argentina (45°S, 68°W) and determined the persistence of *Echinococcus granulosus* eggs in those droppings under natural environmental conditions in that region.

In the first experiment, we analyzed 163 fecal samples collected from urban squares during 8 months time and found parasitic elements in 46.6%. The presence of parasites was independent of the condition of the feces (fresh or dried; $P > 0.05$). Parasites potentially pathogenic in man were present, such as *Toxocara* species (spp.), *Taenia* spp./*Echinococcus* spp., *Uncinarias* spp., and *Entamoeba* spp.

In the second experiment, we analyzed two canine fecal samples contaminated with *E. granulosus* eggs, deposited for 41 months within the natural environment. These parasitic elements persisted during the entire study as attested by light microscopy and the ELISA coproantigen test.

We propose the study of the presence of intestinal parasites in canine feces within the environment as a general strategy for identifying and monitoring areas of risk for canine-related zoonoses since we were able to demonstrate the persistence of *E. granulosus* eggs in deposited canine feces for over 3 years within the area studied.

Keywords: Canine feces; Intestinal parasites; *Echinococcus granulosus*; Environment; Argentina

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1. Introduction

A number of studies have demonstrated the presence of parasites that are pathogenic in man within samples of canine fecal material contaminating urban public areas (Minvielle et al., 1993; Sánchez et al., 1999; Orduna et al., 1999). Eggs of *Taenia* spp. in feces have been shown to survive within the environment for periods up to 300 days at ambient temperatures between 0 and 10 °C and a relative humidity of 85% (Wachira et al., 1991), while those of *Echinococcus multilocularis* did so at temperature extremes of −15 to 27 °C (Veit et al., 1995).

Since the presence and persistence of infectious parasitic forms within urban squares and public recreation areas constitutes a significant health risk (Markell et al., 1986; Minvielle et al., 2000); Uga et al. (1996) have proposed a regulation of the canine population and a control of the contamination of public spaces with the feces of house pets. Accordingly, the objectives of the present study were to screen similar urban areas within the Province of Chubut for the presence of canine fecal material containing intestinal parasites as well as to correlate the frequency of such findings with the characteristics of those feces. Finally, we investigated the persistence of eggs from *Echinococcus granulosus* in particular within canine droppings that had undergone natural aging.

2. Materials and methods

2.1. Study 1

We screened for the presence of intestinal parasites 163 samples of canine fecal material collected at random from 10 squares within the city of Comodoro Rivadavia, Province of Chubut (45°S, 68°W) from November 1999 to June 2000. At the moment of collection, we categorized the feces as fresh if they were of soft and moist consistency or dried out if they were hard, brittle, and light-yellow to white in color. We then kept the samples at 4 °C in 5% (v/v) formaldehyde until further treatment. After homogenization, we processed each specimen according to the techniques of Telemann and Willis (Markell et al., 1999; Martínez Fernandez et al., 2000) and monitored the procedure concurrently in duplicate by light microscopy both with and without Lugol.

We evaluated the resulting data statistically by means of the chi-square independence test and the trial of homogeneity through analysis of proportions. We considered values of $P < 0.05$ to be significant.

2.2. Study 2

In the experimental field of the Hydatidosis Control Program of the Province of Chubut, we screened for eggs and genus specific somatic antigens of adult of *E. granulosus* (Allan et al., 1992; Craig et al., 1995) in canine fecal material deposited onto the surface stratum of soil and subsequently exposed to natural environmental conditions from October 1996 to February 2000. The fecal samples arose from two dogs that were positive for *E. granulosus* and diagnosed at the time of postmortem examination. These dogs harbored a parasitic

burden ranging from 100 to 1000 mature adult of *E. granulosus*, as indicated by counts and optical microscope. In February 2000, we collected the samples and processed them using the techniques of Telemann and Willis (Markell et al., 1999; Martínez Fernandez et al., 2000) in order to screen for eggs; at that time we also carried out the genus specific coproantigen ELISA test for the detection of *Echinococcus* spp., as developed by Allan et al. (1992). In the screening for coproantigens, we first inactivated the feces at −70 °C for 72 h and then left them to hydrate for 24 h after the addition of an equal volume of 0.3% (v/v) Tween in phosphate-buffered saline. After a final 30 min sedimentation at 5135 × *g*, we stored 2.0 ml aliquants of the resulting supernatant at −20 °C until further processing. The cut-off value for the ELISA capture test was 0.215OD ± 3S.D.

With data obtained from the Comodoro Rivadavia Station of the National Meterological Service, we characterized the local regional climate on the basis of an aridity index calculated from the cumulative precipitation value for each season plus the evapotranspiration potential (UNESCO, 1979).

3. Results

3.1. Study 1

Of the 163 samples of canine fecal material studied, 86 (56%) were composed of dry feces, while 77 (44%) contained fresh droppings. Some 76 (46.6%) specimens among the total were positive for intestinal parasites. The distribution of positive and negative samples, however, was independent of the state of hydration of the specimens ($P = 0.33$; Table 1).

Table 2 summarizes the parasitic states and genera of the specimens recovered from the fresh and dry feces. Of the 56 samples with *Toxocara* spp. eggs, 1.6% of the latter contained larvae in phase II in the fresh feces, while 5.4% bore larvae in that phase in the dry feces.

3.2. Study 2

The feces we studied remained exposed to natural environmental conditions for a total of 41 months, while their macroscopical appearance went on varying in accordance with their hydration state from fresh to dry. At the conclusion of that period, we recovered from those feces eggs whose morphology was consistent with that of *E. granulosus*. This identification

Table 1
Characteristics of the feces and the presence or absence of intestinal parasites in 163 canine fecal samples collected from the public squares of Comodoro Rivadavia, Chubut, from November 1999 to June 2000

Feces	Positive	Negative
Dried	37	49
Fresh	39	38
Total	76	87

$P = 0.33$.

Table 2
Frequency (%) of parasite species and parasitic states finding in fresh and dry canine fecal samples collected from the public squares of Comodoro Rivadavia, Chubut, from November 1999 to June 2000

Parasite species	Fresh feces ($n = 77$)	Dry feces ($n = 86$)
Protozoans		
Oocysts		
Isospora spp.	0.93	1.3
Sarcocystis spp.	0.62	–
Cysts		
Entamoeba spp.	3.8	7.6
Giardia spp.	–	0.31
Helminths		
Eggs		
Toxocara spp.	8.2	9.5
Spirocerca spp.	1.6	1.6
Taenia spp. or *Echinococcus* spp.	0.31	2.5
Dipylidium caninum	0.62	–
Uncinaria spp.	0.93	0.62
Larvae of Nematoda[a]	6	12

$N = 163$.
[a]Could be free-living contaminants.

was, in turn, confirmed by a positive result in the ELISA test when we screened the samples for the presence of *Echinococcus* specific coproantigens.

The climate of the region studied proved to correspond to the category of low arid.

4. Discussion

The lack of any correlation between the presence or absence of intestinal parasites within canine feces and the hydration state of the fecal material in which the organisms were found was clearly demonstrated by statistical analysis ($P > 0.05$).

The finding of a greater percentage of larvated eggs from *Toxocara* spp. in the dried feces could be attributed to the requirement for eggs to remain under adequate environmental conditions for larval development. The process of larval maturation to the infectious LII stage within *Toxocara canis* eggs takes from 9 to 18 days at temperatures between 26 and 30 °C (Diez Baños et al., 2000). The temperatures registered during the period studied with an average of 19.2 °C and a range between the extremes of 5.2 and 33.2 °C would result in a development of organisms involving the infectious stages that we describe here.

The etiologic diagnosis of *Dipylidium caninum* can be made through an observation of egg packets (Markell et al., 1999). As a result of the mobility of the segment and of the desiccation process to which environmental feces are subjected, there is a possibility that this structure can become altered and even disappear, a consequence that could affect the identification of *Dipylidium caninum* in these present dried fecal samples.

Chubut province is endemic for *E. granulosus*. It has been estimated that 32 per 100,000 hydatid patiens undergo surgery per year in the province (Eckert et al., 2001). Since 1984, a

control program was implemented. The control measures on the canine population carried out by the hydatidosis monitoring programs in Argentina include the diagnosis and surveillance of echinococcosis in dogs using arecoline bromhydrate (Ministerio de Salud y Acción Social, 1985). The test suffers from several disadvantages: the need for qualified trained personnel, the requirement for specialized equipment and an appropriate work space, the requisite availability of the dogs for several hours, the inefficacy of the test with certain canines, and a degree of risk for the employees as well as the likelihood of contaminating the work area.

By contrast, the results presented here would argue that a study of the prevalence of parasites in canine fecal samples collected from the soil of public places could be considered a simple method for hydatid surveillance that would be applicable to use on an urban scale. The information garnered from such an approach would prove useful for identifying and monitoring areas of risk for zoonotic diseases of parasitic etiology within populated areas.

Willis and Herbert (1984) reported a winter duration period for canine feces within the environment of 2 months. The results of Study 2, furthermore, demonstrates that canine fecal material can last for up to 41 months within the natural environment that we surveyed. Guarnera et al. (2000) estimated that the antigens of *Echinococcus* spp. persist for some 20 days within canine feces under rural conditions in the north of Argentine (Province of Catamarca) and reported that those markers afterwards became preserved within such samples for as much as 13 months in closed containers at 4 °C. Our results further show that parasite specific antigens persist for up to 41 months under low arid climatic conditions.

In a study carried out in Germany, Veit et al. (1995) observed a maximal survival for *E. multilocularis* eggs of 240 days during autumn and winter at an average temperature of 6 °C. In Turkana, Wachira et al. (1991) studied the survival of *E. granulosus* eggs under semiarid environmental conditions. The maximal survival proved to be 19 days in soil and the shade after exposure to an average temperature of 20 °C and a relative humidity of $75 \pm 15\%$ (Wachira et al., 1991). Two observations from our Study 2 are noteworthy here; first, the feces used came from dogs that were parasitized with *E. granulosus*; second, the positive results for *Echinococcus* genus specific antigens with the coproantigen detection ELISA test coincided with our detection under the light microscope of eggs morphologically consistent with those of *E. granulosus* at the end of the experiment. These findings suggest the possibility that eggs from this intestinal parasite can remain within the environment for up to 3 years and 5 months in a low arid climate.

5. Conclusion

The presence of parasites in canine fecal material was independent of whether the feces were fresh or dried out ($P = 0.33$). The dry fecal material contained infectious forms of parasites classified as either potentially or frankly pathogenic for man.

Under low arid climatic conditions, eggs identified both microscopically and immunologically as being from *Echinococcus* spp. persisted within the environment for the entire course of our 41-month study.

The screening of samples of canine feces gathered from the environment for the presence of parasites would constitute a simple and low cost strategy complementing the arecoline

bromhydrate test as an alternative means of monitoring contaminated areas and identifying pathogens for the purpose of implementing control measures.

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