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Short communication

Intestinal parasites of dogs on the Galapagos Islands

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ABSTRACT

Dogs on the Galapagos Islands are a unique population created by isolation from the mainland and regulations prohibiting further importation. The effect of infectious agents of these domestic dogs on the indigenous fauna is largely unknown. The purpose of this study was to determine the prevalence of intestinal parasites in dogs on the Galapagos Islands.

Fecal samples were collected from 97 dogs presented during neutering campaigns on Santa Cruz ($n = 51$), San Cristobal ($n = 17$), and Isabela ($n = 29$) islands. Feces were evaluated for parasites by microscopic examination after zinc sulfate centrifugation flotation as well as by a commercially available IFA for *Cryptosporidium* spp. and *Giardia* spp. Polymerase chain reaction for *Cryptosporidium* spp. DNA and *Giardia* spp. DNA was performed on all positive samples to provide the infecting genotypes.

Ancylostoma caninum (57.7%) and *Toxocara canis* (16.5%) were most commonly detected, followed by *Giardia* spp. (5.2%), *Isospora canis* (4.1%), *Sarcocystis canis* (3.1%), and *Cryptosporidium* spp. (1%). Adequate DNA for sequencing was available for one *Giardia* spp. which was shown to be assemblage D.

Despite being isolated, the dogs on the Galapagos have many of the same enteric parasites detected on the mainland of South America. These dogs are not routinely administered anthelmintics or other drugs, but are often allowed to roam the streets and live in close proximity to humans. Parasite prophylaxis is necessary to decrease the parasite burden within the population and to lessen the risk of spread to humans or other animals also inhabiting the islands.

1. Introduction

The Galapagos Islands is a unique ecosystem separated from mainland Ecuador by approximately 600 miles. It is an archipelago made up of nineteen islands, five of which are inhabited. Despite the unique environment created by this isolation, the Galapagos Islands encounter many of the same problems as seen on mainland South America. Much of this is due to the fact that there has been an influx of visitors and residents (both legal and illegal) to the islands over the past several decades. Tourism has grown at a rate of 14% annually for the last fifteen years (Watkins and Cruz, 2007). The Galapagos' population has dramatically increased over the past several decades as well. The 1972 census revealed a population of 3488; this population expanded to 15,000 in the 1980s and then to 40,000 in 2006. These new visitors and residents often bring with them species foreign to the islands, which can soon become invasive species. For example, the domestic canine is currently classified as an introduced species on the islands.

The majority of the dogs on the islands are owned by residents; however, there is also a significant population of stray dogs that freely roam throughout the towns. Even

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those dogs that are owned are often allowed to roam the streets and beaches during the day and return to the family house at night because few restrictive obligations are placed on dog owners. This behavior creates a public health risk; dogs are allowed to roam throughout the neighborhood, including parks and beaches. Dogs often have access to refuse, can defecate in public areas, and can harass wildlife in town and on the beaches. Such behavior allows for transmission of infectious agents, such as hookworms, roundworms, *Giardia,* and *Cryptosporidium* and promotes the cyclical nature of these infectious within the canine population. The behavior also increases the risk of transmission of zoonotic agents to the local inhabitants and threatens the endemic wildlife that may be susceptible to many of these infectious agents.

As is true with many island populations, there are limitations to the public services such as education and health on the Galapagos, due in large part to the shortage of appropriately trained individuals (Watkins and Cruz, 2007). Veterinary services on the islands are extremely limited and the majority of dogs do not receive prophylactic anthelmintics. Consequently these dogs may have high parasite burdens; the presence of these dogs in close contact with humans may create a high potential risk of zoonotic disease.

While the level of intestinal parasitism in Galapagos dogs is currently unknown, previous studies of other canine disease prevalence in Galapagos dogs have revealed that these dogs have many of the same pathogens as dogs on mainland South America (Levy et al., 2008). The estimates of intestinal parasitism in dogs worldwide vary greatly (Blagburn et al., 1996). This is due to numerous factors that include geographic location, climate, demographic factors, anthelmintic use, status of animal ownership, and diagnostic techniques (Katagiri and Oliveira-Sequeira, 2008). Prevalence rates of intestinal parasites in dogs from mainland South America were 54% in Brazil (Katagiri and Oliveira-Sequeira, 2008), 35.5% in Venezuela (Ramirez-Barrios et al., 2004), 52% in Argentina (Fontanarrosa et al., 2006), and 64.8% in Chile (Lopez et al., 2006). The purpose of this study was to determine the prevalence of intestinal parasites of canines inhabiting the islands of Santa Cruz, San Cristobal, and Isabela within the Galapagos Islands.

2. Materials and methods

Fecal samples were collected from 97 dogs presented during neutering campaigns on Santa Cruz ($n = 51$), San Cristobal ($n = 17$), and Isabela ($n = 29$) islands. All dogs were owned animals and presented to the neuter campaign by owners. Age and breed of the dogs was obtained from a history form completed by the owner at the time of registration. Ages ranged from two months to eighty-four months (seven years) of age. In some instances the age of the dog was unknown by the owner. Many different breeds were represented and none of the dogs had diarrhea at the time of sample collection. Fecal samples (at least 2 g) were obtained at the time of neutering, stored at 4 °C until return to the United States 1–2 weeks after collection, and then stored at 4 °C during analysis. Approximately 2 g of feces were assayed by centrifugal flotation using Sheather's sugar ($\delta = 1.27$) technique followed by microscopic examination for parasites at 100×. Feces were also evaluated for *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts using a commercially available immunofluorescence assay (Merifluor *Cryptosporidium*/*Giardia*, Meridian Diagnostics, Cincinnati, OH). After the slides were stained as instructed by the manufacturer, the samples were examined for the respective cysts and oocysts at 100× and 400× using a fluorescence microscope.

Polymerase chain reaction for *Cryptosporidium* spp. DNA and *Giardia* spp. DNA was performed on all positive samples in an attempt to determine the infecting genotypes. In both the *Cryptosporidium* spp. and Giardia spp. PCR assays the *gdh locus* was targeted following previously published protocols (Scorza et al., 2003; Read et al., 2004). DNA sequences were analyzed in both the forward and reverse directions using an ABI3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequenced *gdh* fragments were compared with those in the Genebank database by BLAST analysis (http://www.ncbi.nlm.nih.gov).

2.1. Statistical analysis

STATA (Stata Statistical Software: Release 10, College Station, TX, USA) was used for the statistical analysis. Descriptive statistics including distribution of dog's age (in months) and proportion of dogs with parasites in each Island, including 95% confidence interval were calculated. The dog's age data were evaluated for normality using the Shapiro–Wilk normality test. Median dog's age was compared between the three islands using the Kruskal–Wallis nonparametric ANOVA. Associations between dog's age, sex, and island location with the prevalence of different parasites were assessed using the Fisher's exact test and logistic regression analysis. Prevalence was calculated as the proportion of tested dogs with positive results.

3. Results

The dogs' age data was not normally distributed (Shapiro–Wilk normality test $p < 0.001$). Thus, median dog's age was compared between the three islands. There was no significant difference in the age distribution between dogs in the three islands ($p = 0.4$; Nonparametric ANOVA (Kruskal–Wallis test)).

The most common parasites detected (Table 1) were *Ancylostoma caninum* (56 dogs; 57.7%) and *Toxocara canis* (16 dogs; 16.5%). *Giardia* spp. were detected in 5 dogs (5.1%; all by IFA), *Isospora canis* in 4 dogs (4.1%), *Sarcocystis canis* in 3 dogs (3.1%), and *Cryptosporidium* spp. in 1 dog (1%).

The risk of *Ancylostoma* infection was significantly influenced by island location (Fisher's exact test, $p = 0.037$). Logistic regression analysis indicated that dogs on San Cristobal island had a significantly lower risk of infection than dogs on Santa Cruz (OR = 0.25, $p = 0.021$, OR 95% CI = 0.08–0.81). However, dogs on Isabela did not have a significantly lower risk of infection than dogs on Santa Cruz (OR = 1.12; $p = 0.8$, OR 95% CI = 0.43–2.93). There was no

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Table 1
Overall prevalence of parasites in dogs by island.

Island/parasite	*Ancylostoma caninum*	*Toxocara canis*	*Giardia* spp.	*Isospora canis*	*Sarcocytis canis*	*Cryptosporidium* spp.	Total # dogs parasitized
Santa Cruz ($n = 51$)	32 (62.7%)	6 (11.8%)	3 (5.9%)	3 (5.9%)	2 (2.9%)	1 (1.9%)	36 (70.6%)
Isabela ($n = 29$)	19 (65.5%)	6 (20.7%)	0 (0%)	1 (3.4%)	1 (3.45%)	0 (0%)	21 (72.4%)
San Cristobal ($n = 17$)	5 (29.4%)	4 (23.5%)	2(11.8%)	0 (0%)	0 (0%)	0 (0%)	8 (47.1%)
Total ($n = 97$)	56 (57.7%)	16 (16.5%)	5 (5.1%)	4 (4.1%)	3 (3.1%)	1 (1.1%)	65 (71.4%)

Table 2
Distribution of parasites by sex.

	A. caninum	*T. canis*	*Giardia* spp.	*I. canis*	*Sarcocytis canis*	*Cryptosporidium* spp.
Female ($n = 55$)	31	8	3	2	1	1
Male ($n = 42$)	25	8	2	2	2	0
Total ($n = 97$)	56	16	5	4	3	1

significant difference for risk of infection with *T. canis* between the three islands (Fisher's exact test, $p = 0.38$). Statistical analysis for the remaining four parasites was performed and indicated there was no significant difference for risk of infection between the three islands, but the results are of limited value due to the small number of dogs with these parasites.

The prevalence of *Ancylostoma* and *Toxocara*, the two most commonly detected parasites, was not influenced by age of the dog ($p = 0.49$, 95% CI = 0.96–1.01 for *Ancylostoma*; $p = 0.24$, 95% CI = 0.92–1.02 for *Toxocara*). Statistical analysis for the remaining four parasites was performed and indicated the prevalence was not influenced by age, but the results are of limited value due to the small number of dogs with these parasites.

Distribution of the positive samples by sex is presented in Table 2. There was no significant difference on the prevalence of *Ancylostoma* or *Toxocara* according of the sex of the dogs (Fisher's exact test $p = 0.84$ for *Ancylostoma*; $p = 0.59$ for *Toxocara*). Statistical analysis for the remaining four parasites was performed and indicated there was no significant difference on the prevalence according to sex of the dogs, but just as with the results in regards to age these findings are of limited value due to the small number of dogs with these parasites.

The number of different parasites detected per dog was also calculated by island (Table 3). The majority of dogs, 53.6%, had only one parasite detected. There was no significant differences between islands on the proportion of dogs in which 1 parasite was detected (Fisher's exact test $p = 0.44$), two parasites were detected ($p = 0.52$), greater than two parasites were detected ($p = 1.00$) or in dogs without any parasites detected ($p = 0.17$).

4. Discussion

These results indicate that despite their isolation, dogs on the Galapagos Islands have been exposed to and harbor the parasites commonly seen in mainland South America. This is most likely due to the fact that the dogs originated from the mainland and were first brought to the islands by early settlers and visitors, so these parasites were present prior to strict control on interisland movement of dogs. However, there continues to be a significant problem of illegal smuggling of dogs onto the islands, which has the potential to continually introduce infected dogs to the islands.

As mentioned previously, the age of dogs included in this study ranged from two months to seven years of age. While the upper limit of age limit of seven years may appear young, there are most likely several reasons for this age limit. First, dogs were presented to the spay-neuter clinic by their owners and it is possible these owners may have elected not have surgery performed on older pets, thus influencing the sample population. The age limit may also be influenced by the state of veterinary care on the islands. There are few veterinary services available on the islands; consequently, very few dogs receive any type of preventive care and are rarely treated unless they are presented with an illness which may have influenced survival to older age groups.

Several concerns arise from the level of parasitism indicated in this study. The first relates to public health as many of the parasites detected are zoonotic. The current lifestyle of dogs often allows for free roaming throughout the town during the day; this can lead to contamination of the environment with canine excrement. Since many of these parasites are passed through the fecal oral route, this leads to a continual parasitism cycle within the canine

Table 3
Number of parasites detected in dogs by island.

	No parasites detected	Dogs with 1 parasite	Dogs with 2 parasites	Dogs with >2 parasites
Santa Cruz ($n = 51$)	15 (29.4%)	30 (58.8%)	5 (9.8%)	1 (2%)
Isabela ($n = 29$)	8 (27.6%)	15 (51.7%)	5 (17.2%)	1 (3.4%)
San Cristobal ($n = 17$)	9 (52.9%)	7 (41.2%)	1 (5.9%)	0 (0%)
Total ($n = 97$)	32 (33%)	52 (53.6%)	11 (11.3%)	2 (2.1%)

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population and the chance for transmission to humans as well. Also, because veterinary care is extremely limited and there is apparently little awareness of zoonotic disease, this increases the risk of disease transmission.

No samples were collected from stray dogs on the islands. However, since the majority of owned dogs are allowed to roam throughout the towns during the day, there is a high likelihood these dogs would come into contact with any stray dogs that may also share the same local environment. Since neither owned nor stray dogs are treated with anthelmintics, they most likely have a similar parasite burden; therefore the findings of this study, which are representative of owned dogs, can be extrapolated to the stray dog population with confidence.

Another concern arising from the level of parasitism indicated by this study is the risk to the indigenous wildlife of the islands. The Galapagos is the last of the archipelagos that still maintain 95% of its biodiversity (Watkins and Cruz, 2007). Consequently it is extremely important that this ecosystem be protected to maintain this biodiversity. Previous introduced species have had dramatic impact on the endemic species of the islands. When goats were introduced to the islands they cleared vegetation and were a significant contributing factor to the loss of all but one of the tortoises of Pinta Island (www.darwinfoundation.org). Cats were also introduced and are now classified as an invasive species which threatens the avian species of the islands, including the Darwin finches. Dogs can harbor the canine distemper virus, some strains of which are transmissible to marine mammals (Di Guardo et al., 2005).

Cryptosporidium and *Giardia* have both also been found to infect several marine mammal species, including harp seals, hooded seals, harbor seals, and the California sea lion (Appelbee et al., 2005; Deng et al., 2000). Both *Cryptosporidium* and *Giardia* have also been found in marine waters (Johnson et al., 1995) and shellfish have been shown to harbor these two organisms (Fayer et al., 2004). Knowing that dogs on the Galapagos are free to roam the beaches, it can be assumed that if they are infected with either of these organisms, water contamination could occur. Since these organisms can remain infective in the marine water for several weeks (Appelbee et al., 2005), dogs could indirectly serve as a source of infection for marine mammals. The limited home range of many of these marine mammals also contributes to their harboring of these organisms. Most of the marine mammals do not travel far from their individual island habitat (Wolf and Trillmich, 2007); consequently they are at increased risk of infection the longer they remain in potentially contaminated waters. These mammals could also serve as reservoirs for environmental transmission of these protozoa, including reinfection of the canine population which freely roam the beaches of the islands.

While no specific protozoal surveillance has been conducted in the Galapagos marine mammals, the Galapagos sea lions are most closely related to the California sea lion. There may be differences secondary to habitat, seasonality, or differences in diet (Deng et al., 2000) between the two species; however, it is possible that Galapagos sea lions may be infected by the protozoa just as the California sea lions are. The marine mammals can function as indicators of contamination of marine water with these pathogens. So while the pathogens may not directly affect the marine mammals, they are an indicator of pathogens that are potentially harmful to the local human population that often use the same water for a variety of activities.

In conclusion, veterinary care and public health education need to be increased in order to protect the dogs, their owners, and the native species of the Galapagos Islands. The dogs of the islands are exposed to many of the same enteric parasites found on the mainland. Many of these parasites are readily susceptible to common anthelmintics. Routine prophylactic use of these anthelmintics would aid in the control of these zoonotic pathogens. Stricter regulations enforcing dog ownership would aid in decreasing the public health threat and the threat to native species. Without implementation of public health education and routine veterinary care, the dogs will continue to serve as a reservoir of parasites and threaten human and native species health.

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