Short Report: Multiple Zoonotic Pathogens Identified in Canine Feces Collected from a Remote Canadian Indigenous Community

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Abstract. Five genera of potentially zoonotic bacteria and parasites were detected in environmentally collected fecal samples from a remote indigenous community in Northern Saskatchewan, Canada. Organisms identified include *Toxocara canis*, *Echniococcus granulosus*, *Giardia duodenalis*, *Cryptosporidium* spp., and *Campylobacter* spp. The prevalence and intensity of *Giardia* spp. and *Campylobacter* spp. in fecal samples was particularly remarkable. Three-quarters of samples tested contained at least one zoonotic species of *Campylobacter*, and *C. jejuni*-containing feces had an average of 2.9×10^5 organisms/g. Over one-half of samples tested contained *Giardia* spp. with an average of 9,266 cysts/g. Zoonotic *G. duodenalis* Assemblage A was the only *Giardia* spp. genotype identified. These data suggest that canine feces have the potential to pose a significant health risk to Canadians in rural and remote indigenous communities.

Domestic dogs have long been recognized to be a potential source of zoonoses for people.[1–4] In particular, zoonotic bacteria and parasites harbored in the canine intestine have been shown to pose a significant risk to human health.[1–4] People are exposed to these pathogens through direct or indirect contact with infected dogs or their feces, and they may become infected after inadvertent ingestion of a zoonotic agent.[2–4]

In Canada, indigenous people living in rural and remote communities seem to have an increased risk of exposure to and infection with certain canine fecal zoonoses compared with other Canadians.[5–7] This may be related to the fact that many of these communities have large populations of free-roaming domestic dogs and little access to veterinary care. These dogs have frequent contact with one another, canine feces, and a variety of refuse and foodstuffs that potentially contain zoonotic agents, all of which promote intestinal infection with a variety of zoonoses and subsequent human exposure.

Despite the apparent zoonotic risks that domestic dogs may pose to indigenous Canadians, there are very few contemporary studies that characterize the microbial and parasite content of canine feces in these communities. This is problematic, because people infected with canine fecal zoonoses often exhibit non-specific clinical signs[6,8,9] that can be misdiagnosed if health care workers are unaware of the presence of these pathogens in their jurisdictions. Also, until the health risk posed by domestic dogs is better understood, it will not be possible to institute effective strategies to prevent human infection.

It is also important to consider that exposure to canine fecal zoonoses could present a more significant health problem in indigenous communities compared with other Canadian populations. Indigenous peoples seem to be at increased risk for certain infectious diseases, including those caused by zoonotic pathogens,[5,7] likely because of traditional practices as well as risk factors associated with poverty, including poor nutrition and substandard housing.[5,10] Also, infectious diseases may have a more significant impact on the health of indigenous people compared with other Canadians because of concurrent health problems and decreased access to health care.[10]

In 2008, a 6-year-old girl from a remote indigenous community in Northern Saskatchewan was diagnosed with an *Echinococcus granulosus* parasitic infection that was most likely acquired through contact with canine feces.[11] The ensuing, community-based epidemiologic investigation revealed widespread exposure to and infection with *E. granulosus* in humans and dogs, respectively,[11] creating concern that other zoonotic pathogens might be harbored by dogs in the community. To investigate this possibility, environmentally collected canine fecal samples were screened for a variety of bacterial and parasitic zoonoses.

One block was randomly selected within each of the three distinct neighborhoods that comprise the main community. All yards on that block were surveyed on foot, and any canid feces found were collected in individual plastic bags. Fecal samples were also collected from around the community landfill based on the researchers' suspicion that domestic dogs might frequent the landfill to scavenge on garbage. A total of 155 samples were collected from the four study sites. During the fecal-collection procedure, researchers observed numerous free-ranging dogs throughout the community, despite recent depopulation attempts. A numerical estimate of past or present dog populations could not be obtained.

Samples were subdivided, and subsamples were sent to the World Health Organization Collaborating Center for the Molecular Epidemiology of Parasitic Infections (Murdoch University, Murdoch, Australia) and the University of Saskatchewan (Saskatoon, Canada) where they were analyzed for the presence of *E. granulosus* as previously described.[11]

At the University of Saskatchewan, subsamples were also analyzed using quantitative fecal-flotation[12] and sucrose-gradient[13] techniques to concentrate and enumerate parasite eggs and *Giardia* spp. cysts/*Cryptosporidium* spp. oocysts, respectively.

To determine the predominant *Giardia* spp. genotypes, polymerase chain reaction (PCR) was performed on selected samples as previously described[14] to amplify a segment of the *G. duodenalis* β-giardin gene. Samples that contained > 10,000 *Giardia* spp. cysts/g were selected for analysis, because these feces had the potential to cause the greatest environmental

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contamination with *Giardia* spp. A total of 19 samples with > 10,000 cysts/g had sufficient material available for analysis. Four samples with 1,000–10,000 cysts/g were also tested to evaluate the sensitivity of the PCR assay at our institution. PCR was performed on DNA extracted from sucrose gradient concentrates using the QIAGEN DNeasy Blood and Tissue Kit (QIAGEN Inc., Valencia, CA). Because *G. duodenalis* is the only *Giardia* spp. known to infect dogs[9,15] and all samples were observed to contain *Giardia* spp. cysts on microscopic examination, any failure to amplify product was interpreted to be the result of poor sample integrity or test sensitivity. Any product obtained by PCR was sequenced using the amplification primers. Sequencing of the β-giardin gene allows *G. duodenalis* samples to be classified into groups of genotypes called assemblages.[14] This classification is essential when determining the zoonotic potential of *Giardia* spp. found in canine feces, because dogs may be infected with assemblages A, B, C, and D, of which only A and B are known to infect humans.[14–16] It should be noted that the genus *Cryptosporidium* contains several species with zoonotic potential.[15,16] The *Cryptosporidium* spp. identified in this study were not identified to the species level; however, domestic dogs have the potential to become infected with *C. parvum* and *C. canis*, both of which are known to cause disease in people.[15–17]

A subset of 60 fecal samples, which was comprised of 20 randomly selected samples from each of the three neighborhoods in the community, was selected for total bacterial DNA extraction (QIAGEN Stool Kit; QIAGEN Inc., Valencia, CA). These samples were tested for the presence of 14 known species of *Campylobacter* using a cpn60-based real-time quantitative PCR[18] (also conducted at the University of Saskatchewan). Bacterial culture was not performed because of financial constraints and the degraded state of many of the samples.

Distribution of pathogen-containing fecal samples and relative intensity of infection were compared among study sites using the χ^2, Wilcoxon rank sum, and Kruskall–Wallis tests.[19] All calculations were performed using STATA/IC 10.0 (StatCorp LLP, College Station, TX) with a significance level of $P < 0.05$.

Five genera of potentially zoonotic pathogens were found in 155 canine fecal samples collected within a northern Saskatchewan indigenous community (Table 1). Of the 24 *Giardia* spp.-containing samples analyzed by PCR, 13

(56%) produced amplicons of the expected size. Sequencing revealed that the DNA amplified from all 13 (100%) samples belonged to the zoonotic group of *G. duodenalis* genotypes known as assemblage A.[9,14–16] Of the 60 samples tested for *Campylobacter* spp., 28 (47%) contained one or both of the established zoonotic species *C. jejuni* and/or *C. upsaliensis*.[8,20]

There was no significant difference in the prevalence of *Toxocara canis*-, *Giardia* spp.-, *Cryptosporidium* spp.-, *C. jejuni*-, or *C. upsaliensis*-containing fecal samples between study sites. The prevalence of *E. granulosus* was significantly greater in neighborhoods 2 and 3 compared with neighborhood 1 and the landfill ($P = 0.005$). This could be the result of differences in the prevalence of *E. granulosus* infection among the different canid groups that frequent and/or populate the four sites, although the reason for these differences could not be determined.

The results of this study show that canine feces within this community contain a variety of zoonotic organisms that could pose a health risk to people coming into contact with dogs or their excrement. Zoonotic agents identified include bacteria, protozoa, and helminths known to cause both systemic and gastrointestinal disease in people. *T. canis* and *E. granulosus* are the causative agents of larval migrans and cystic hydatid disease, respectively,[2,4,6] whereas *G. duodenalis* assemblage A, *Cryptosporidium* spp., and *Campylobacter* spp. are responsible for diarrheal diseases in people.[9,16,20] Previous reports have implicated domestic dogs as a potential source of these zoonoses.[2,6,9,11,15,21]

In this study, the prevalence of *T. canis* in fecal samples was greater than that previously identified in owned dogs in the United States and Canada,[17,22,23] although it was within the range reported for stray dogs[24,25] and dogs in northern Canadian aboriginal communities.[1,26] This variation in prevalence of infection could be the result of differences in anthelmintic treatment among the different groups of dogs. The prevalence of *E. granulosus*-containing feces in this study was also within the range reported for dogs in northern Canadian aboriginal communities.[1,26] However, the prevalence of *E. granulosus*-infected dogs in these communities is highly variable among geographic locations and over time, likely as a result of variation in the dietary composition of dogs. The prevalence of *Cryptosporidium* spp. in fecal samples was similar to that identified in dogs in other North American studies.[17,22]

TABLE 1
Prevalence and intensity of multiple zoonotic organisms identified in environmentally collected canine fecal samples from an indigenous Canadian community

Pathogen	Prevalence*					Intensity†			
	Neighborhood 1	Neighborhood 2	Neighborhood 3	Landfill	Total	Minimum	Median	Mean	Maximum
Helminths									
Toxocara canis	12/48 (33%)	6/43 (16%)	6/25 (24%)	2/39 (5%)	26/155 (17%)	3	80	826	6,250
Echinococcus granulosus	1/48 (2%)	5/43 (12%)	3/25 (12%)	0/39 (0%)	7/155 (5%)	4	15	22	75
Protozoa									
Giardia spp.	28/48 (58%)	32/43 (74%)	15/25 (60%)	20/39 (51%)	95/155 (61%)	33	2,200	9,266	> 55,000
Cryptosporidium spp.	2/48 (4%)	2/43 (5%)	0/25 (0%)	1/39 (3%)	5/155 (3%)	5	18	29	68
Bacteria									
Campylobacter spp.	17/20 (85%)	15/20 (75%)	13/20 (65%)	na	45/60 (75%)	na	na	na	na
Campylobacter jejuni	6/20 (30%)	5/20 (25%)	9/20 (45%)	na	20/60 (33%)	1.6×10^4	9.6×10^4	2.9×10^5	2.3×10^6
Campylobacter upsaliensis	8/20 (40%)	7/20 (35%)	5/20 (25%)	na	20/60 (33%)	6.0×10^3	5.0×10^4	8.6×10^5	1.3×10^7

*Proportion of fecal samples tested that contained the zoonotic organism of interest.
†Number of infectious units per gram of feces (1 infectious unit = 1 egg for *T. canis* and *E. granulosus*, 1 cyst for *Giardia* spp., 1 oocyst for *Cryptosporidium* spp., and 1 bacterium for *Campylobacter* spp.)

Of particular note is the prevalence and intensity of *Giardia* spp. and *Campylobacter* spp. in these fecal samples. The prevalence of *Giardia* spp. was much higher than expected given that the reported prevalence of *Giardia* spp. infection in stray and owned dogs in Canada and the United States is usually less than 10%,[17,22–25,27] and the reported prevalence of infection in dogs from two northern Canadian aboriginal was not greater than 33%.[1] In this study, well over one-half of the samples collected contained *Giardia* spp. cysts, and PCR results indicated that all samples in which product could be amplified contained zoonotic *G. duodenalis* assemblage A. On average, the fecal samples in this study contained over 9,000 cysts/g (mean) with 25% and 3% of samples containing > 10,000 and > 50,000 cysts/g, respectively. Because the infectious dose for *G. duodenalis* in humans is thought to be as low as 10 cysts,[28] it is reasonable to consider that canine feces have the potential to be a significant source of *Giardia* spp. for people in this community.

This may also be the case for *Campylobacter* spp., because three-quarters of the fecal samples tested contained potentially zoonotic species of this bacterium. A previous study in Ontario, Canada did not identify *Campylobacter* spp. in a group of healthy dogs using PCR.[22] However, studies of healthy dogs in the United Kingdom and Ireland, also using PCR, have shown a high prevalence of infection (upwards of 40% in some cases) with both *C. jejuni* and *C. upsaliensis*,[29,30] similar to what was found in this study. The potential *Campylobacter* spp.-related zoonotic risk associated with canine feces is also supported by the intensity of infection in many of the samples. For example, the infectious dose of *C. jejuni* for people is thought to be approximately 800 organisms,[20] and the *C. jejuni*-positive samples in this analysis contained 20–30,000 times that many organisms per gram of feces.

It is interesting to note that, for all organisms identified in this study, the mean intensity of infection was consistently greater than the median (Table 1). This could reflect an aggregated distribution of infectious organisms within this dog population. In other words, a small number of dogs may harbor the majority of the organisms, and the remainder of the population has a much lower intensity of infection. This would increase the mean intensity of infection relative to the median. Although the aggregated distribution described above is most commonly associated with parasitic metazoa,[31] similar variation between the median and mean were observed for all organisms identified in this study, suggesting that a similar phenomenon could occur with protozoa and bacteria. This seems to be the case for *Giardia* spp. in this study (Figure 1). Although some feces contained over 50,000 *Giardia* spp. cyst/g, over one-half of the samples contained < 5,000 cysts/g, suggesting that certain dogs with a heavier pathogen burden are responsible for a greater degree of environmental contamination compared with others. This suggestion is further supported by the fact that, although there was no significant difference in distribution of *T. canis*-containing fecal samples among study areas, the average intensity of infection was significantly higher in neighborhoods 1 and 3 compared with the landfill (*P* = 0.009 and 0.02, respectively). The apparently aggregated distribution of canine fecal zoonoses in this community has future research and management implications, because it highlights the importance of identifying the most heavily infected animals to properly assess and manage the risk of human exposure.

A limitation of this study is the fact that prevalence of infection in dogs could not be definitively determined with the sam-
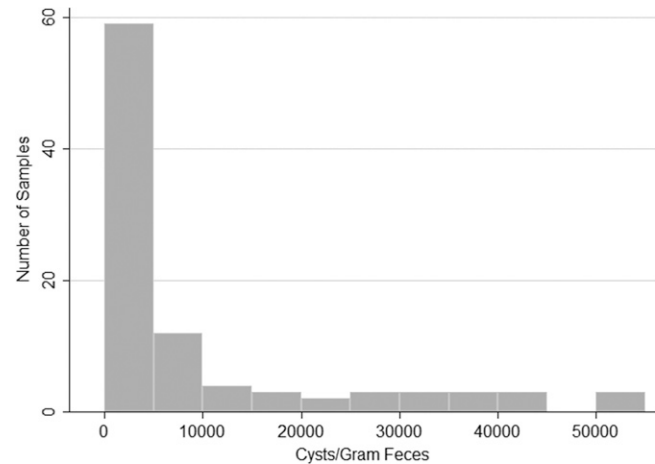

Figure 1. Summary of the intensity of infection with *Giardia* spp. in environmentally collected canine fecal samples from an indigenous Canadian community.

pling methodology used (i.e., environmentally collected canine fecal samples), because feces could not be traced back to the animal of origin and multiple samples may have originated from a single dog. For this reason, it is difficult to compare these results with those of studies that that describe the prevalence of fecal zoonoses in dogs. However, this study does confirm that dogs in this community are infected with a number of zoonoses and provides a crude evaluation of the degree of environmental contamination with these organisms. In this situation, the prevalence and distribution of zoonosis-containing fecal samples (versus prevalence of infection in dogs themselves) may, in fact, provide a more accurate assessment of the potential for human exposure to canine fecal zoonoses. Previous studies have indicated that soil contamination with zoonotic parasites is a risk factor for human infection,[32] and there is reason to believe that, in this community, people are most likely to be exposed to canine fecal zoonoses in the environment because of limited direct contact between people and free-roaming dogs.[11]

Overall, this study revealed the presence of a number of zoonotic bacteria and parasites in environmentally collected canine feces from a remote Canadian indigenous community. There is evidence to suggest that, in this community, contact with canine feces has resulted in human exposure to and infection with at least one zoonotic pathogen (*E. granulosus*).[11] To date, no other cases of infection with canine fecal zoonoses have been definitively identified; however, other than the *Echinococcus* investigation,[11] no studies have been undertaken to determine the prevalence of these organisms and/or their associated diseases in people from this community. Given the generally non-specific clinical signs caused by infection with canine-fecal zoonosis,[6,8,9] it is possible that human infection with these organisms has occurred and gone undiagnosed. Because the pathogens identified in this study pose a potential threat to human health, animal and human health-care professionals working in rural and remote indigenous Canadian communities should be aware of the significant and ongoing public-health risks associated with domestic dogs.

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