

GIARDIA AND CRYPTOSPORIDIUM SPECIES AND GENOTYPES IN COYOTES (*CANIS LATRANS*)

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Abstract: Feces and duodenal scrapings were collected from 22 coyotes (*Canis latrans*) killed in managed hunts in northeastern Pennsylvania. Polymerase chain reaction (PCR) methods were used to detect *Giardia* and *Cryptosporidium* spp. PCR-amplified fragments of *Giardia* and *Cryptosporidium* spp. SSU-rRNA genes were subjected to DNA sequence analysis for species/genotype determination. Seven coyotes (32%) were positive for *G. duodenalis*: three assemblage C, three assemblage D, and one assemblage B. Six coyotes (27%) were positive for *Cryptosporidium* spp. One isolate shared 99.7% homology with *C. muris*, whereas five others (23%) shared 100% homology with *C. canis*, coyote genotype. This is the first report on multiple genotypes of *Giardia* spp. in coyotes and on the prevalence of *Cryptosporidium* spp. genotypes in coyotes.

Key words: *Canis latrans*, coyote, *Cryptosporidium*, genotypes, *Giardia*, prevalence.

INTRODUCTION

Giardia duodenalis (syn. *G. lamblia* and *G. intestinalis*) and *Cryptosporidium* spp. are commonly identified intestinal parasites of mammals, including humans.^{1,12} However, identification of animal sources of *Giardia* spp. cysts or *Cryptosporidium* spp. oocysts that are infectious for humans has been complicated by the fact that different species or genotypes within these genera are often morphologically indistinguishable, even though genetic and biologic differences exist.^{1,12} Molecular analysis is essential for identifying species and genotypes and characterizing their host ranges.^{6,7,15} For *G. duodenalis*, assemblages A and B have the widest host ranges, infecting humans and a variety of other animals, including dogs. The remaining assemblages have restricted host ranges: C and D infect dogs, E infects hoofed livestock, F infects cats, and G infects rats.⁷ In the case of *Cryptosporidium* species, *C. hominis* generally exhibits a human-to-human transmission cycle, although animal infections have been reported.⁸ The primary zoonotic species is *C. parvum*, although *C. canis*, *C. felis*, *C. meleagridis*, *C. muris*, *C. suis*, and *Cryptosporidium* cervine genotype have zoonotic potential as well.¹⁵

There are abundant reports of both *Cryptosporidium* and *Giardia* spp. in wildlife, and wild animals have been considered potential reservoirs of zoonotic organisms; however, reports based solely on microscopy do not provide information on the potential risk of transmission to humans or domes-

tic animals. Recently, molecular studies have shown that wildlife can harbor both host-adapted and zoonotic strains of both *Cryptosporidium* and *Giardia* spp.^{11,18}

Thus, although early studies document the presence of both parasites in wildlife, molecular analysis to identify species or genotypes is essential to determine whether they represent a risk of infection to humans or domesticated animals.

The population and range of the coyote (*Canis latrans*) have been expanding in the northeastern United States for many decades. Their increasing numbers increase the risk of human and domestic animal contact with coyote feces, via contaminated pastures, water, and recreational areas.

Although species of *Giardia* have been reported in coyotes,^{3,4,9} only a previous report from our laboratory⁹ attempted to identify the species and genotypes, indicating that the coyote isolate shared 98.8% homology at the β -giardin gene with an isolate from a domestic dog (subsequently classified as assemblage C). *Cryptosporidium canis*, coyote genotype, has been reported in coyotes; however, prevalence data were not obtained.¹⁷ Therefore, the current study was undertaken to determine the prevalence of *Giardia* and *Cryptosporidium* species and genotypes in coyotes.

MATERIALS AND METHODS

Coyote feces and tissues

Coyotes were killed by a trappers' association in northeastern Pennsylvania. This managed hunt was conducted in 2004 for population control. The hunting area included nine counties in the northeastern corner of the state. Two counties bordered the state of New York and two bordered New Jersey. Twenty-two coyote carcasses were brought to a central

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processing location, where fecal samples were collected from the rectum and gut scrapings were taken from the proximal duodenum. Feces from each animal were placed in plastic specimen cups with lids. Duodenal scrapings (~100 μ l) were also obtained from each animal and placed in 1.5-ml centrifuge tubes containing 180 μ l of DNA extraction buffer. All cups and tubes were labeled to identify the animal source, placed on ice, and transported to the Environmental Microbial Safety Laboratory at Beltsville, Maryland, where they were stored at 4°C and processed within 5 days.

Processing of fecal samples

Fifteen grams of feces from each coyote was placed into a 50-ml centrifuge tube, capped, and thoroughly mixed with 35 ml dH₂O. The fecal suspension was passed through a brass screen with 45- μ m-diameter openings, collected into a second 50-ml tube, and adjusted to a final volume of 50 ml with deionized water. The tubes were labeled, capped, and centrifuged at 1,500 g for 15 min, the supernatant discarded, and the fecal pellet resuspended in 25 ml dH₂O. Twenty-five milliliters of CsCl (1.4 g/ml) was added to each tube and the samples were thoroughly mixed. The samples were subjected to a second centrifugation at 250 g for 20 min. Following the second centrifugation, the top 4 ml of supernatant was aspirated from each sample and transferred into a 15-ml tube. Deionized H₂O was added to all tubes to bring the final volume to 15 ml. Samples were washed twice with dH₂O, and the final pellet was suspended in 500 μ l of dH₂O.

Molecular analysis of samples

For DNA extraction, total DNA was extracted from each CsCl-cleaned fecal sample or duodenal scraping using a DNeasyTissue Kit (Qiagen, Valencia, California 91355, USA) with a slight modification to the manufacturer's protocol supplied with the kit. This protocol utilized reagents provided by the manufacturer. A total of 50 μ l of sample was suspended in 180 μ l of ATL buffer and thoroughly mixed by vortexing. To this suspension, 20 μ l of proteinase K (20 mg/ml) was added, and the sample was thoroughly mixed. Following an overnight incubation of the mixture at 55°C, 200 μ l of AL buffer was added. The remaining protocol followed manufacturer's instructions with one exception. To increase the quantity of recovered DNA, the nucleic acid was eluted in 100 μ l of AE buffer.

To detect species and genotypes of *Giardia* and *Cryptosporidium*, separate two-step nested polymerase chain reaction (PCR) protocols were used to amplify a fragment of the respective SSU-rRNA

gene as previously described.^{5,16} PCR products were analyzed on 1% agarose gel and visualized by ethidium bromide staining.

All positive samples were prepared and subjected to DNA sequence analysis. PCR products were purified using the EXO-SAP enzyme kit (USB Corporation, Cleveland, Ohio 44128, USA). Purified products were sequenced with the same PCR primers used for the second amplification in 10 μ l reactions, Big Dye chemistries, and an ABI3100 sequencer analyzer (Applied Biosystems, Foster City, California 94404, USA). Each sample was sequenced in both directions. Sequence chromatograms from each strand were aligned and inspected using Lasergene software (DNASTAR, Inc., Madison, Wisconsin 53715, USA).

RESULTS

Twenty-two coyotes (12 males and 10 females) were available for necropsy. Their weights ranged from 31 to 44 lbs and averaged 35 lbs. The animals generally appeared healthy and well fed, although some had hair loss and skin lesions characteristic of acariasis (mange).

A sample was considered *Giardia* spp.-positive if either the feces or the duodenal scraping tested positive. Only fecal samples were analyzed for *Cryptosporidium* spp. because this parasite would not normally be found in the duodenum.

Giardia spp. was detected in seven of 22 coyotes (32%). Of the seven positive samples, sequence analysis indicated that 14% were assemblage C, 14% were assemblage D, and 4.5% were assemblage B. Six of 22 coyotes (27%) were infected with *Cryptosporidium* spp. Sequence analysis revealed that one sample (4.5%) shared 99.7% homology with *C. muris*. The remaining five samples (23%) were *C. canis*, coyote genotype.

Sequences were deposited in GenBank under accession numbers DQ385545–DQ385549.

DISCUSSION

Much of the prevalence data on species of *Cryptosporidium* and *Giardia* in wildlife predates the use of molecular testing and thus provides no information on the risk of transmission of these parasites to humans or domesticated animals. Although recent studies have begun to incorporate molecular methods to determine species and genotypes found in wild animals, most of this research, including the current study, is limited in scope because of the inherent difficulty in sampling wildlife populations.

Species of *Cryptosporidium* and *Giardia* have been reported in numerous wildlife hosts, including coyotes,^{3,17,18} in which *C. canis*, coyote genotype,

has been reported. Previous reports of *Giardia* spp. in coyotes are limited to microscopic identification, with the exception of work in our laboratory,⁹ in which an isolate obtained from a coyote was found to share 98.8% homology with an isolate obtained from a dog (subsequently identified as assemblage C). Thus, the current study demonstrates for the first time the presence of three *Giardia duodenalis* assemblages, B, C, and D, in the coyote along with the presence of *C. canis*, and also provides prevalence data on both parasites in a localized coyote population. The finding of assemblages C and D is not surprising in that these genotypes are routinely found in dogs. The finding of assemblage B, however, could represent either an actual infection or organisms from an infected animal that was eaten by the coyote and remained in the gastrointestinal tract at the time our specimen was collected. Assemblage B has been reported in dogs, lending weight to the possibility that this was an actual infection. However, beavers often harbor assemblage B¹¹ and could certainly serve as a food source for coyotes.

The presence of *C. canis*, coyote genotype, previously reported in coyotes, is confirmed in the present study. The current data indicate that almost one fourth of the animals in this area are infected with this genotype. Because *C. muris* has not been reported in canids, the presence of this species in a single coyote is most likely the result of consumption of an infected rodent, although the possibility of an active infection cannot be completely discounted. However, as suggested for *Giardia* spp., the *Cryptosporidium* spp. detected could have resulted from food sources.

This study identifies for the first time the presence of multiple *Giardia* genotypes in coyotes and provides prevalence data on those genotypes as well as on *Cryptosporidium*. All coyotes from which samples were collected were considered adults; it is not known whether the prevalence and genotypes of these parasites would be the same in neonatal or juvenile animals. Previous research conducted on dairy cattle revealed age-related changes in prevalence of both *Cryptosporidium* spp. and *Giardia* spp.,^{2,10,13,14} as well as changes in *Cryptosporidium* spp. genotypes¹⁰. It is possible that such age-related differences could occur in coyotes as well. Additionally, the animals that had acariasis may have been immunosuppressed.

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