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Host-Adapted *Cryptosporidium* spp. in Canada Geese (*Branta canadensis*)

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The prevalence and distribution of *Cryptosporidium* spp. in the fecal droppings of the free-living waterfowl Canada geese were examined at 13 sites in Ohio and Illinois. On the basis of the analysis of the small-subunit rRNA gene by PCR, followed by restriction fragment length polymorphism analysis and DNA sequencing, 49 (23.4%) of 209 fecal specimens collected from 10 sites (76.9%) were positive for *Cryptosporidium* spp. The following five *Cryptosporidium* species and genotypes were identified: *Cryptosporidium* goose genotype I (in 36 specimens), *Cryptosporidium* goose genotype II (in 9 specimens), *Cryptosporidium* duck genotype (in 1 specimen), *Cryptosporidium parvum* (in 4 specimens), and *C. hominis* (in 2 specimens). *Cryptosporidium* goose genotype I was the most prevalent parasite and was found at all five *Cryptosporidium*-positive sites in Ohio and at four of five positive sites in Illinois, followed by *Cryptosporidium* goose genotype II, which was found at two of five positive sites in Ohio and at four of five positive sites in Illinois. *Cryptosporidium* goose genotype II was detected for the first time, and it is phylogenetically related to goose genotype I and the duck genotype. All three genotypes have not so far been reported in humans, and their pathogenicity in geese has not been determined. Only 10.2% of the *Cryptosporidium*-positive specimens had *C. parvum* and *C. hominis*. The results of this study indicate that Canada geese might only serve as accidental carriers of cryptosporidia infectious to humans and probably play a minor role in the animal-to-human transmission cycle of the pathogen.

The Canada goose population in the United States has been increasing in the last decade. Most Canada geese are migratory, wintering in the United States and migrating to their summer breeding grounds in Canada (21). However, the availability of suitable habitats, such as grassy areas with bodies of water in urban and suburban areas, has increased the numbers of these geese that have become year-round residents in the United States. Their feces litter the ground in many public areas such as parks, golf courses, cemeteries, and residential areas. That these Canada geese might carry and distribute human pathogens is a major public health concern (2, 4, 6, 10, 11).

Cryptosporidia are among the pathogens potentially disseminated by Canada geese (6, 9, 10). *Cryptosporidium parvum* oocysts have been shown to retain viability and infectivity following passage through Canada geese (5). It has been suggested that migratory Canada geese might disseminate infectious oocysts of *C. parvum* through their feces into public water sources (6). This is of particular significance because Canada geese prefer aquatic habitats and contaminated water is a major source of human *Cryptosporidium* infection in the United States (3). In recent years, laboratory-confirmed cases of waterborne cryptosporidiosis have increased significantly in the United States (3).

Many *Cryptosporidium* species are present in animals, of which *C. parvum* and *C. hominis* are the most important causes of cryptosporidiosis in humans (12, 23). *C. hominis* was previously known as *C. parvum* human genotype or genotype 1, infects mostly humans, and is presumably transmitted from humans to humans. *C. parvum* infects both ruminants and humans and can be acquired by both human-to-human and zoonotic transmission pathways (14, 18). Lately, some other *Cryptosporidium* species and genotypes have also been found in both immunocompetent and immunocompromised persons, including *C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, and *Cryptosporidium* pig and cervine genotypes (1, 7, 15–20, 22, 23). Many other host-adapted *Cryptosporidium* spp. have also been found in a variety of animal species, including farm animals, pets, and wildlife (24).

The number of *Cryptosporidium* species in Canada geese is not known. Only one study has indicated that Canada geese might play a role as mechanical carriers of infectious *C. parvum* (6). Another study reported a host-adapted *Cryptosporidium* sp. (goose genotype) in the feces of migratory Canada geese (24) that so far has not been found in humans. Cryptosporidiosis is apparently very common in Canada geese because cryptosporidia (species and genotypes undetermined) were found in a recent study in feces of Canada geese at 9 of 10 sites in the Toledo, Ohio, area (10).

The purpose of this study was to determine if Canada geese are sources of *C. parvum* and *C. hominis*, which are the dominant species infecting humans. Feces of Canada geese were collected from 13 sites in Ohio and Illinois and examined for the prevalence and distribution of *Cryptosporidium* species by PCR analysis of the small-subunit (SSU) rRNA gene. Results of this study have shown the presence of two host-adapted

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Cryptosporidium genotypes in Canada geese, with only occasional detection of human-pathogenic *Cryptosporidium* spp. in the feces of these animals.

MATERIALS AND METHODS

Specimen collection and DNA extraction. Canada goose fecal specimens were collected from seven sites in Toledo, Ohio, and six sites in DuPage County, Ill. To reduce the chance of one animal contributing to more than one specimen, only recently deposited feces, appearing wet and loose and warm to the touch, were collected from each sampling site. In some cases, specimens were collected immediately after Canada geese were seen defecating. Each fecal specimen contained about 1 g of feces and was placed in a vial containing 2.5% potassium dichromate solution. A total of 209 individual specimens, of which 148 (70.8%) were from Ohio and 61 (29.2%) were from Illinois, were collected and analyzed for *Cryptosporidium* species and genotypes as described below. The majority (>90%) of the birds were Canada geese. However, a small number of ducks and other waterfowl were seen in some of the sites. Most of the Canada geese were adults, with only a few juveniles. The specimens from Illinois were collected in July 1999, whereas the specimens from Ohio were collected between July and October 2002. Most of the birds were migratory, because by the end of October or the beginning of November there were only few Canada geese at most of the study sites.

A portion of about 200 mg from each specimen was washed twice in a Microfuge tube with 1 ml of distilled water and centrifugation at 12,000 × *g* for 15 min. No oocyst concentration or purification and no microcopy were done before DNA extraction. The pellet was treated initially with 66.7 μl of 1 M KOH and 18.6 μl of 1 M dithiothreitol, followed by neutralization with 8.6 μl of 25% (vol/vol) hydrochloric acid. The DNA lysate was then extracted once with phenol-chloroform-isoamyl alcohol (25:24:1) solution, and genomic DNA was extracted with the QIAamp DNA Stool Mini Kit (QIAGEN Inc., Valencia, Calif.) as previously described (24). Extracted DNA was stored at −70°C prior to being analyzed.

Differentiation of *Cryptosporidium* spp. by PCR-RFLP. *Cryptosporidium* species and genotypes were determined by a previously described technique based on PCR-restriction fragment length polymorphism (RFLP) analysis of the SSU rRNA gene (22, 23). In this method, a fragment of 826 to 864 bp of the SSU rRNA gene was amplified by nested PCR. For the detection and differentiation of *Cryptosporidium* species and genotypes, 10 μl of the secondary PCR product was subjected to restriction digestions with SspI (New England BioLabs, Beverly, Mass.) and VspI (GIBCO BRL, Grand Island, N.Y.). Differences in SspI and VspI banding patterns in 2% agarose electrophoresis were used in the determination of *Cryptosporidium* species or genotypes (24, 25). Each DNA specimen was analyzed at least three times by PCR-RFLP with 0.5, 1.0, or 2.0 μl of DNA as the template. Positive (*C. serpentis* DNA) and negative (no template DNA) controls were included in each PCR run.

DNA sequencing and phylogenetic analysis. At least two independent PCR products from each positive specimen were sequenced in both directions with an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, Calif.). The sequences obtained were aligned with each other and those of known *Cryptosporidium* spp. with the ClustalX software (http://inn-prot.weizmann.ac.il/software/ClustalX.html). A neighbor-joining tree was generated with the TREECON software (http://www.psb.rug.ac.be/bioinformatics/psb/Userman/treeconw.html) on the basis of genetic distances calculated by the Kimura two-parameter model. The reliability of branches was assessed by bootstrap analysis with 1,000 replicates.

Nucleotide sequence accession numbers. The unique partial SSU rRNA sequences generated in this study have been deposited in the GenBank database under accession no. AY504512 to AY504517.

RESULTS

***Cryptosporidium* spp. in feces of Canada geese.** Of the 209 fecal specimens examined in this study, 49 (23.4%) were positive for cryptosporidia by the SSU rRNA-based PCR assay used. The real *Cryptosporidium* prevalence rate in Canada geese could have been underestimated, because only 200 mg of fecal specimen was analyzed for each bird and no oocyst concentration was determined prior to DNA extraction. RFLP analysis of PCR products with SspI and VspI showed three banding patterns. Most of the specimens had only one RFLP banding pattern. Three samples, however, had mixed RFLP patterns, indicating the presence of more than one *Cryptosporidium* genotype. Altogether, the banding pattern of the previously identified *Cryptosporidium* goose genotype (24) was seen in 45 specimens, the pattern of *C. parvum* was seen in 5 specimens, and the pattern of *C. hominis* was seen in 2 specimens.

Because some *Cryptosporidium* spp. are known to have similar RFLP banding patterns, all PCR products were sequenced to verify the species or genotype identity. Two types of DNA sequences were obtained from the 45 specimens that showed the SspI and VspI banding pattern for the *Cryptosporidium* goose genotype; 36 (80%) specimens produced sequences similar or identical to the previously identified *Cryptosporidium* goose genotype (24), which is now renamed *Cryptosporidium* goose genotype I, and 9 (20%) specimens produced sequences for a new *Cryptosporidium* genotype, which is now named *Cryptosporidium* goose genotype II. Of the five specimens with the *C. parvum* banding pattern, four produced sequences identical or similar (with a 1- or 2-bp difference) to *C. parvum*, and one produced sequences identical to the previously identified *Cryptosporidium* duck genotype (13). All sequences obtained from the two specimens with the *C. hominis* banding pattern were identical to those of *C. hominis*.

Of these positive specimens, most (46 of 49 or 93.9%) had only one *Cryptosporidium* genotype. Three specimens, however, had two *Cryptosporidium* genotypes each: one specimen (no. 6846) had both goose genotype I and the duck genotype, one specimen (no. 6869) had both goose genotype I and *C. parvum*, and one specimen (no. 6888) had both *C. parvum* and *C. hominis* (Table 1 and Fig. 1).

Distribution of *Cryptosporidium* spp. Of the seven sites in Ohio investigated, five (71.4%) were positive for the *Cryptosporidium* goose genotypes and two of the five sites were also positive for *C. parvum* and *C. hominis*. One goose at a site in Ohio was also positive for the *Cryptosporidium* duck genotype. Of the six sites in Illinois, five (83.3%) were positive for cryptosporidia, all of which belonged to *Cryptosporidium* goose genotype I or II. The percentages of specimens positive for cryptosporidia ranged from 0 to 80% in Ohio and from 0 to 50% in Illinois (Table 1). *Cryptosporidium* goose genotype I was the most widely distributed, being detected at all five of the positive sites in Ohio and at four of five positive sites in Illinois, followed by *Cryptosporidium* goose genotype II, which was detected at four of five positive sites in Illinois and at two of five positive sites in Ohio.

Genetic relationships among *Cryptosporidium* spp. in Canada geese. Among the five *Cryptosporidium* spp. and genotypes identified in Canada geese, the SSU rRNA gene of *Cryptosporidium* goose genotypes I and II and the duck genotype showed high sequence homology to each other, as reflected by direct sequence alignment, as well as genetic distance calculation. Nucleotide differences of the SSU rRNA gene among the three genotypes were 2.4 to 3.1%. In contrast, the differences between these genotypes and *C. parvum* or *C. hominis* were 4.5 to 7.1% (Table 2). As expected, the differences between *C. parvum* and *C. hominis* were very small (<1.0%). There were minor sequence variations (1 bp each) within both *Cryptosporidium* goose genotypes I and II.

Phylogenetic analysis confirmed the close relatedness among *Cryptosporidium* goose genotypes I and II and the duck geno-

TABLE 1. Detection of cryptosporidia in fecal specimens from Canada geese

State and site no.	No. of positive specimens/total (% positive)	No. of infections	*Cryptosporidium* genotype and/or spp. (no. positive)
Ohio			
1	2/12 (17)	2 single	Goose genotype I (1) Goose genotype II (1)
2	3/17 (18)	3 single	Goose genotype I (3)
3	0/21 (0)	0	None
4	0/18 (0)	0	None
5	1/40 (3)	1 single	Goose genotype I (1)
6	15/25 (60)	13 single 2 double	Goose genotype I (14) Duck genotype (1) *C. parvum* (2)
7	12/15 (80)	11 single 1 double	Goose genotype I (7) Goose genotype II (2) *C. hominis* (2) *C. parvum* (2)
Illinois			
1	3/10 (30)	3 single	Goose genotype I (2) Goose genotype II (1)
2	0/10 (0)	0	None
3	3/7 (43)	3 single	Goose genotype II (3)
4	1/10 (10)	1 single	Goose genotype I (1)
5	2/10 (20)	2 single	Goose genotype I (1) Goose genotype II (1)
6	7/14 (50)	7 single	Goose genotype I (6) Goose genotype II (1)
Total (13 sites)	49/209 (24)[a]	46 single 3 double	5 *Cryptosporidium* species and genotypes

[a] Ten of 13 sites.

type. These three parasites clustered together in a neighbor-joining tree constructed with sequences from all known *Cryptosporidium* spp. (24), with a sequence of *Eimeria tenella* (accession no. AF026388) as the outgroup, with 89% bootstrap support (data not shown). As expected, *C. parvum* and *C. hominis* were related and formed a cluster together. Among *Cryptosporidium* goose genotypes I and II and the duck genotype, the two goose genotypes were closely related, forming a group within the cluster, with high bootstrap support. There were two subdivisions in both the goose genotype I and II groups (Fig. 1).

DISCUSSION

The results of this study indicate that multiple *Cryptosporidium* species and genotypes are found in Canada geese. In addition to goose genotypes, other *Cryptosporidium* spp., such as the duck genotype, *C. hominis*, and *C. parvum*, were detected in the feces of Canada geese. *Cryptosporidium* goose genotype I had been previously detected (24), but *Cryptosporidium* goose genotype II had not been seen before and was detected in the feces of Canada geese for the first time. No other published study has identified the presence of more than one *Cryptosporidium* species or genotype in Canada geese. This study demonstrates that Canada geese can carry cryptosporidia of goose, duck, human, and ruminant origins.

TABLE 2. Genetic distances among *Cryptosporidium* species and genotypes in Canada geese

Genotype or species (specimen no.)	No. of nucleotide differences/100 bases from specimen no.:				
	6754	888	6876	6888	6869
Goose genotype I (6754)	0.00	2.94	3.13	7.05	6.86
Goose genotype II (888)		0.00	2.39	6.07	6.26
Duck genotype (6876)			0.00	5.10	4.52
C. hominis (6888)				0.00	0.90
C. parvum (6869)					0.00

Cryptosporidium goose genotypes I and II were the most common *Cryptosporidium* spp. in the fecal specimens studied; they had prevalence rates of 17.2 and 4.3%, were detected at 9 and 6 of the 13 study sites, respectively, and constituted 91.8% of the positive specimens. The high occurrence of these two *Cryptosporidium* spp. indicates that they are likely true parasites of Canada geese. Another, related *Cryptosporidium* sp., the duck genotype (13), was found in one goose together with goose genotype I. It remains to be determined whether this parasite is infectious to Canada geese. The two other species, *C. parvum* and *C. hominis*, were only found in five geese, reaffirming the previous conclusion that oocysts of these two species were merely passing through the digestive tracts of foraging Canada geese without establishing infection (5). When this paper was under revision, an SSU rRNA sequence characterization of eight *Cryptosporidium*-positive specimens in a recent study showed the presence of five *Cryptosporidium* genotypes in Canada geese in the United States (8). Three of the *Cryptosporidium* genotypes belonged to goose genotypes I (geese 1, 2, 3a, 6, and 8) and II (goose 9) and the duck

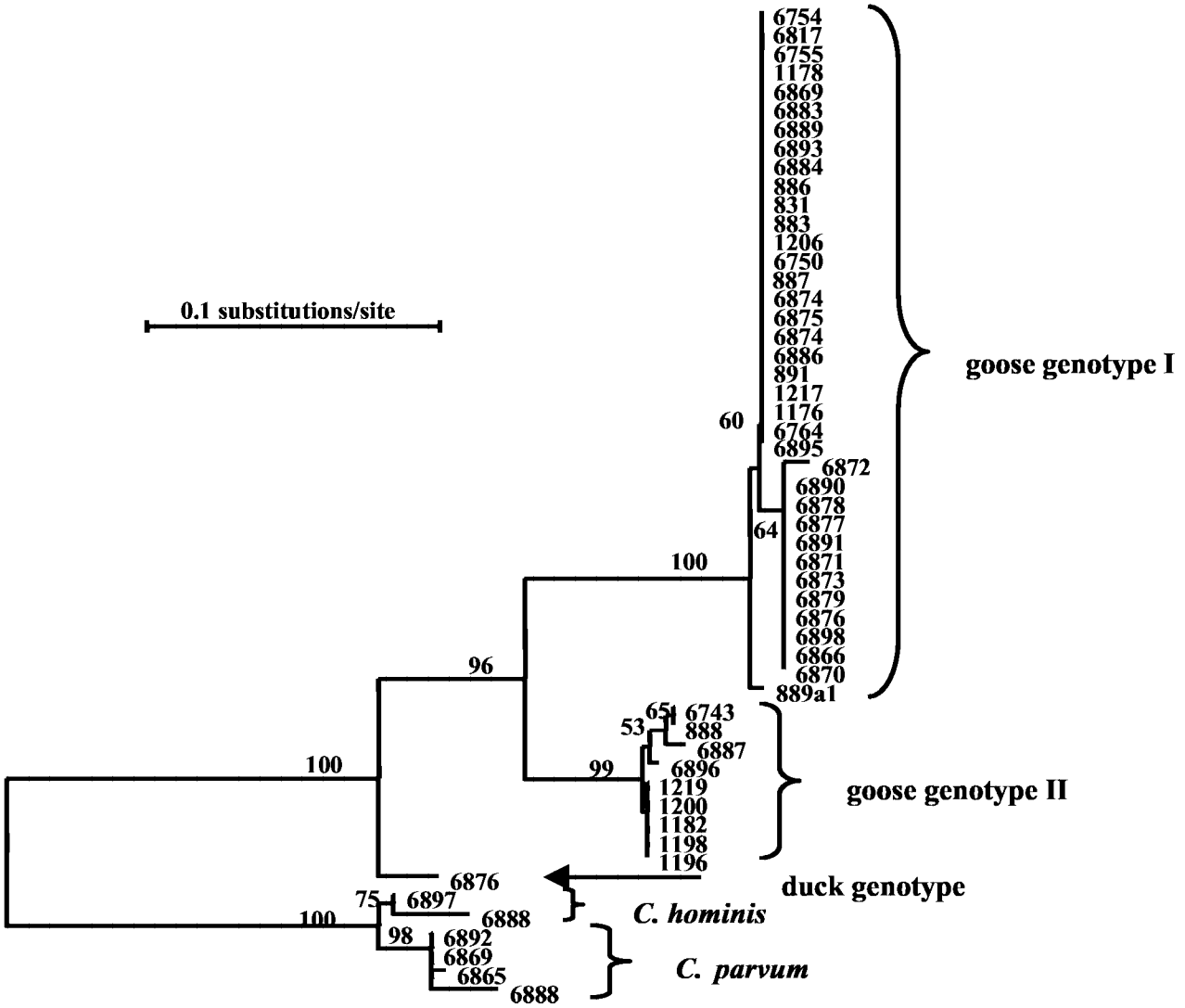

FIG. 1. Phylogenetic relationships among *Cryptosporidium* species and genotypes from Canada geese based on SSU rRNA sequences. The Kimura two-parameter model was used for distance calculation. Numbers on branches are percent bootstrap values from 1,000 resamplings.

genotype (goose 5) in the present study, whereas the remaining two genotypes (geese 3b and 7) represented new *Cryptosporidium* genotypes.

For both Ohio and Illinois, all of the sites within each state are within a 20-mile radius of one another, but three sites were negative for cryptosporidia, while two sites in Ohio were positive for *C. hominis* and *C. parvum* in addition to the two goose genotypes. All of the study sites are either used for recreational activities or close to institutional buildings. However, the two sites in Ohio positive for *C. hominis* and *C. parvum* were much more intensely used than the other sites and often were littered with garbage. It is plausible that *C. hominis* and *C. parvum* oocysts in a few geese were acquired locally from the environment contaminated by human activities, rather than mechanically carried oocysts picked up by Canada geese at distant locations.

C. hominis and *C. parvum* are the important pathogenic species in humans and are responsible for most of the cryptosporidiosis outbreaks worldwide (12, 18, 20). Humans and ruminants are the principal sources of these pathogens. Since sites used by Canada geese also serve as residential and/or recreational facilities, there is potential for human contact with the feces of this waterfowl. However, in this study few goose fecal specimens (5 of 209 or 2.4%) were positive for *Cryptosporidium* spp. that are pathogenic to humans, indicating that Canada geese might serve only as a minor source of infection in humans. Most of the specimens were positive for *Cryptosporidium* goose genotype I, which so far has not been seen in humans. Further research is required to determine whether similar distributions of *Cryptosporidium* species and genotypes also occur in Canada geese in other areas and settings.

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