APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1998, p. 2736–2738 Vol. 64, No. 7
0099-2240/98/$04.00+0

Giardia sp. Cysts and Infectious *Cryptosporidium parvum* Oocysts in the Feces of Migratory Canada Geese (*Branta canadensis*)

THADDEUS K. GRACZYK,[1]* RONALD FAYER,[2] JAMES M. TROUT,[2] EARL J. LEWIS,[3] C. AUSTIN FARLEY,[3] IRSHAD SULAIMAN,[4] AND ALTAF A. LAL[4]

Department of Molecular Microbiology and Immunology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland 21205[1]; Immunity and Disease Resistance Laboratory, Livestock and Poultry Science Institute, Agricultural Research Services, U.S. Department of Agriculture, Beltsville, Maryland 20705[2]; Cooperative Oxford Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Oxford, Maryland 21654[3]; and Department of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Chamblee, Georgia 30341[4]

Received 18 March 1998/Accepted 21 April 1998

Fecal droppings of migratory Canada geese, *Branta canadensis*, collected from nine sites near the Chesapeake Bay (Maryland), were examined for the presence of *Cryptosporidium parvum* and *Giardia* spp. *Cryptosporidium* sp. oocysts were found in feces at seven of nine sites, and *Giardia* cysts were found at all nine sites. The oocysts from three sites were infectious for mice and molecularly identified as the zoonotic genotype of *Cryptosporidium parvum*. Waterfowl can disseminate infectious *C. parvum* oocysts in the environment.

Two genotypes of *Cryptosporidium parvum*, the animal-adapted (or zoonotic) and human-adapted genotypes, have been identified by molecular techniques (1, 12, 14); both genotypes can produce life-threatening infections in immunocompromised and immunosuppressed people. The infectivity of *Cryptosporidium* oocysts is long lasting (3). Aquatic birds have been incriminated in the contamination of water with microbiological agents (11, 16). Oral inoculations of Canada geese (*Branta canadensis*) and ducks (*Anas platyrhynchos*) with infectious *C. parvum* oocysts demonstrated that the oocysts passed through the gastrointestinal tract, retaining their infectivity (8, 10). This suggested that waterfowl had the potential to serve as mechanical carriers of *C. parvum* and to disseminate infectious oocysts in the environment (2). *Giardia* spp. are protozoan pathogens of vertebrates; their infectious stage, the cyst, is transmitted via water and fecal-oral contamination (18). Successful transmission of avian isolates of *Giardia* to mammals may have serious implications for contamination of watersheds (17). The purpose of the present study was to determine if *Cryptosporidium* oocysts or *Giardia* cysts might be present in the fecal droppings of free-ranging Canada geese, and if so, to assess the concentration of these cystic stages in the goose feces. An additional goal was to determine the infectivity for mammals of any *Cryptosporidium* oocysts that were recovered from feces of geese, including molecular characterization of the genotype.

Freshly deposited fecal droppings of *B. canadensis* were collected from nine locations of the Chesapeake Bay (Maryland) area (Table 1) between October and November 1997. Each of nine pooled droppings from a single flock of birds was weighed. Each of the collective fecal specimens was separately processed (5). The fecal sediments (5) were subjected to cesium chloride gradient centrifugation (5) and processed by the cellulose acetate membrane-filter dissolution method for recovery of *Cryptosporidium* oocysts (7). The method has a recovery efficiency of 78.8% (9) and does not alter the infectivity of *C. parvum* oocysts (9). *Giardia* sp. cysts and *Cryptosporidium* sp. oocysts were visualized with the immunofluorescent antibody of the MERIFLUOR *Cryptosporidium*/*Giardia* test kit (Meridian Diagnostics, Cincinnati, Ohio) and counted. Equal fractions of 3 suspensions of *Cryptosporidium* sp. oocysts recovered from feces collected at Wye Island (Table 1) were pooled, and each of four 9-day-old suckling BALB/c mice was inoculated with approximately 9.0×10^4 oocysts (3). The mice were euthanatized 96 h postinoculation, and their intestines were processed (3). Histologic sections were examined for developmental stages of *Cryptosporidium* (3). The remainder of the oocyst pool was subjected to molecular genotyping utilizing PCR for *Cryptosporidium* TRAP C2 and beta-tubulin genes (14).

The mean weight of an individual fecal dropping ± standard deviation was 17.2 ± 1.9 g (range, 13.5 to 21.6 g). The total weight of feces collected was 12.6 kg (approximately 733 droppings). Oocysts of *Cryptosporidium* sp. were recovered from goose feces at seven of the nine sites (Table 1). Oocysts of *Cryptosporidium* sp. recovered from feces collected at Wye Island (Table 1) induced severe infections in all four mice. Results of PCR confirmed that the oocysts used in the mouse bioassay were of genotype 2, the animal-adapted, or zoonotic, genotype of *C. parvum*. The mean concentration of oocysts ± standard deviation was 370 ± 197 oocysts/g (Table 1). *Giardia* sp. cysts were recovered from all nine pooled fecal specimens. The concentration of *Giardia* sp. cysts was 405 cysts/g (Table 1). Comparison of the paired numbers of *Giardia* sp. cysts with *Cryptosporidium* oocysts in feces showed that the concentration of *Giardia* sp. cysts was significantly higher than the concentration of *Cryptosporidium* oocysts (Sign test; $P < 0.02$). The concentrations of cystic stages of both pathogens were correlated with each other (Spearman rank correlation; $r = 0.69$; $P < 0.01$). Significant correlation was observed between the weight of fecal specimens and the number of recovered oocysts (Spearman rank correlation; $r = 0.50$; $P < 0.04$).

Visitation of gulls at drinking and recreational water reservoirs caused a decline in the microbiological quality of such waters (11). Although viable oocysts of *Cryptosporidium* sp.

* Corresponding author. Mailing address: Department of Molecular Microbiology and Immunology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD 21205. Phone: (410) 614-4984. Fax: (410) 955-0105. E-mail: tgraczyk@jhsph.edu.

TABLE 1. Numbers and concentrations of *Giardia* sp. cysts, *Cryptosporidium* sp. oocysts, and infectious *C. parvum*[a] oocysts recovered from fecal droppings collected in the Chesapeake Bay (Maryland) area from nine flocks of migratory Canada geese (*B. canadiensis*)

Site of fecal dropping collection			Total wt of fecal droppings (kg)	*Cryptosporidium* oocysts		*Giardia* cysts	
Name	Latitude (N)	Longitude (W)		Total no. recovered (10^6)	Concn (per g)	Total no. recovered (10^6)	Concn (per g)
Grasonville[b]	38°56.886′	76°13.709′	1.79	0.12	67	0.31	173
Oxford 1[c]	38°41.120′	76°09.654′	1.60	0	0	0.12	75
Oxford 2[c]	38°41.052′	76°09.717′	0.91	0.31	341	0.54	593
Perrys Corner[d]	38°56.090′	76°11.523′	0.83	0	0	0.37	446
Bryatown[d]	38°56.692′	76°10.311′	1.34	0.37	276	0.51	381
Carmichael[d]	38°56.858′	76°08.226′	1.82	0.71	390	0.97	533
Wye Island 1[a,e]	38°53.848′	76°08.952′	1.17	0.62	530	0.92	786
Wye Island 2[a,f]	38°53.803′	76°09.360′	1.91	1.31	686	0.38	199
Wye Island 3[a,f]	38°53.628′	76°09.774′	1.23	0.37	301	0.56	455

[a] *Cryptosporidium* sp. oocysts identified by PCR and mouse bioassay as the zoonotic (animal-adapted) strain of *C. parvum*.
[b] Meadow near pond.
[c] Soybean stubble.
[d] Corn stubble.
[e] Standing soybeans.
[f] Standing corn and/or corn stubble.

were recovered from gull feces the organism was not identified to the species level (16). Gulls can be infected with *Cryptosporidium baileyi* (13). The species of oocysts recovered previously from gull droppings (16) is unknown, and therefore the epidemiological importance of gulls in transmitting *C. parvum* remains unknown. In contrast, the present study demonstrated for the first time the presence of infectious oocysts of *C. parvum* in the fecal droppings of birds, migratory Canada geese, as well as the presence of *Giardia* sp. cysts in these feces. *Cryptosporidium parvum* is unable to establish intestinal infection in birds (8, 10), and therefore we conclude that the infectious *C. parvum* oocysts were acquired by the birds from their natural habitat. The mouse bioassay and PCR showed that the oocysts recovered from goose feces collected at Wye Island (Table 1) were viable and infectious and represented the zoonotic genotype of *C. parvum*. Interestingly, the same genotype of *C. parvum* has been recovered from oysters in the Wye River near Wye Island (3). The Eastern Shore of Maryland is a predominantly agricultural region with scattered cattle farms, and migratory geese were actually observed to wander behind the cattle and pick up undigested corn from their feces. Previous studies showed that approximately 25% of *C. parvum* oocysts administered orally to geese and ducks was recovered (8, 10); thus, geese in the present study may actually ingest higher numbers of the oocysts than we recovered. Because avian giardiasis produces a minimal number of cysts (4) and this pathogen was not previously reported from the Canada goose, it is possible that *Giardia* cysts were acquired by the birds in their natural habitat. It has been suggested that aquatic birds such as herons be considered as reservoirs for human waterborne giardiasis (4). Avian isolates of *Giardia* cysts established severe infections in mice, and it has been concluded that birds can contaminate waterways with *Giardia* cysts and that these cysts are potentially a danger for humans, livestock, and native animals (17). Irrespective of the origin of *Giardia* sp. cysts in the goose feces (indigenous infection versus a mechanical carrier), these cysts produced positive immunofluorescent-antibody reactions with the commercial test kit used for testing of raw water samples (6, 15). The present study provides clear evidence that birds can act as mechanical carriers of infectious oocysts of *C. parvum* and can disseminate these oocysts in the environment, including drinking water supplies.

We thank the Wye Island Natural Resources Management Area (Queenstown, Md.) and the Wildfowl Trust of North America (Grasonville, Md.) for facilitating collection of feces of migratory Canada geese and K. N. Greenhawk (COLMAP, Oxford, Md.) for providing geographical location data. We acknowledge C. Carpenter for technical assistance.

This study was supported in part by Maryland Sea Grant R/F-88.

REFERENCES

1. **Awad-El-Kariem, F. M., H. A. Robinson, D. A. Dyson, D. Evans, S. Wright, M. T. Fox, and V. M. McDonald.** 1995. Differentiation between human and animal strains of *Cryptosporidium parvum* using isoenzyme typing. Am. J. Trop. Med. Hyg. **52:**559–564.
2. **Fayer, R., T. K. Graczyk, C. A. Farley, E. J. Lewis, and J. M. Trout.** 1997. The potential role of waterfowl and oysters in the complex epidemiology of *Cryptosporidium parvum*, p. 153–158. *In* C. R. Fricker, J. L. Clancy, and P. A. Rochelle (ed.), 1997 International Symposium on Waterborne *Cryptosporidium* proceedings. American Water Works Association Press, Denver, Colo.
3. **Fayer, R., T. K. Graczyk, E. J. Lewis, J. M. Trout, and C. A. Farley.** 1998. Survival of infectious *Cryptosporidium parvum* oocysts in seawater and Eastern oysters (*Crassostrea virginica*) in the Chesapeake Bay. Appl. Environ. Microbiol. **64:**1070–1074.
4. **Georgi, M. E., M. S. Carlisle, and L. E. Smiley.** 1986. Giardiasis in a great blue heron (*Ardea herodias*) in New York state: another potential source of waterborne giardiasis. Am. J. Epidemiol. **123:**916–917.
5. **Graczyk, T. K., and M. R. Cranfield.** 1997. Assessment of the conventional detection of fecal *Cryptosporidium serpentis* oocysts in subclinically infected captive snakes. Vet. Res. **27:**185–192.
6. **Graczyk, T. K., M. R. Cranfield, and R. Fayer.** 1996. Evaluation of commercial immunoassay (EIA) and immunofluorescent antibody (IFA) test kit for detection of *Cryptosporidium* oocysts other than *Cryptosporidium parvum*. Am. J. Trop. Med. Hyg. **54:**274–279.
7. **Graczyk, T. K., M. R. Cranfield, and R. Fayer.** 1997. Recovery of waterborne oocysts of *Cryptosporidium* from water samples by the membrane-filter dissolution method. Parasitol. Res. **83:**121–125.
8. **Graczyk, T. K., M. R. Cranfield, R. Fayer, and M. S. Anderson.** 1996. Viability and infectivity of *Cryptosporidium parvum* oocysts are retained upon intestinal passage through a refractory avian host. Appl. Environ. Microbiol. **62:**3234–3237.
9. **Graczyk, T. K., R. Fayer, M. R. Cranfield, and R. Owens.** 1997. *Cryptosporidium parvum* oocysts recovered from water by the membrane filter dissolution method retain their infectivity. J. Parasitol. **83:**111–114.
10. **Graczyk, T. K., M. R. Cranfield, R. Fayer, J. Trout, and H. J. Goodale.** 1997. Infectivity of *Cryptosporidium parvum* oocysts is retained upon intestinal passage through a migratory waterfowl species (Canada goose, *Branta canadensis*). Trop. Med. Int. Health **2:**341–347.
11. **Hatch, J. J.** 1996. Threats to public health from gulls (*Laridae*). Int. J. Environ. Health Res. **6:**5–16.
12. **Morgan, U. M., C. C. Constantine, P. O'Donoghue, B. P. Meloni, P. A. O'Brien, and R. C. A. Thompson.** 1995. Molecular characterization of *Cryptosporidium* isolates from humans and other animals using random amplified polymorphic DNA analysis. Am. J. Trop. Med. Hyg. **52:**559–564.
13. **Pavlasek, I.** 1993. The black-headed gull (*Larus ridibundus* L.), a new host

for *Cryptosporidium baileyi* (Apicomplexa: Cryptosporidiidae). Vet. Med. **38:** 629–638.
14. **Peng, M. M., L. Xiao, A. R. Freeman, M. J. Arrowood, A. A. Escalante, A. C. Weltman, C. S. L. Ong, W. R. MacKenzie, A. A. Lal, and C. B. Beard.** 1997. Genetic polymorphism among *Cryptosporidium parvum* isolates: evidence for two distinct human transmission cycles. Emerg. Infect. Dis. **3:**567–573.
15. **Smith, H. V., and J. B. Rose.** 1998. Waterborne cryptosporidiosis: current status. Parasitol. Today **14:**14–22.
16. **Smith, H. V., J. Brown, J. C. Coulson, G. P. Morris, and R. W. A. Girdwood.** 1993. Occurrence of oocysts of *Cryptosporidium* sp. in *Larus* spp. gulls. Epidemiol. Infect. **110:**135–143.
17. **Upcroft, J. A., P. A. McDonnell, A. N. Gallagher, N. Cheng, and P. Upcroft.** 1997. Lethal *Giardia* from a wild-caught sulphur-crested cockatoo (*Cacatus galerita*) established in vitro chronically infects mice. Parasitology **114:**407–412.
18. **Wolfe, M. S.** 1992. Giardiasis. Clin. Microbiol. Rev. **5:**93–100.