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Survey of Canada goose feces for presence of *Giardia*

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Abstract: As resident Canada goose (*Branta canadensis*) populations increase throughout North America, so do the health and environmental risks associated with goose feces. Previous studies suggest that goose feces may be a conduit for transmitting *Giardia*, a protozoan that is parasitic to humans. We surveyed fecal droppings from free-ranging resident Canada geese for *Giardia* spp. at 9 sites in the Triangle area (Raleigh, Durham, and Chapel Hill) of North Carolina in 2007 and 2008. Samples (n = 234) were tested using the ProSpect® *Giardia* EZ Microplate Assay, and there were no positives. Our results indicate that risk of zoonotic giardiasis from Canada goose feces in the Triangle area of North Carolina is low.

Key words: *Branta canadensis*, Canada geese, *Giardia* spp., human–wildlife conflicts, zoonotic diseases

Populations of resident Canada geese (*Branta canadensis*) have increased in North America over the past 50 years, especially in suburban areas (Conover and Chasko 1985, Ankney 1996). Corporate and residential growth has generated an increased number of artificial ponds and lakes surrounded by maintained turfgrass that create suitable habitat for resident (nonmigratory) Canada geese (Conover and Chasko 1985). High concentrations of resident geese in suburban areas, particularly where people are frequent visitors, increase the possibility of human and pet contact with goose feces (Graczyk et al. 2008). Also, geese commonly use areas adjacent to waterways, which are either secondary sources of drinking water or recreational areas for people (Conover and Chasko 1985). Increased exposure to goose feces may potentially lead to the transmission of infectious diseases to wildlife, livestock, pets, and people (Graczyk et al. 2008).

Feces of Canada geese have been shown to contain *Cryptosporidium parvum, Escherichia coli*, and *Giardia* spp. (Graczyk et al. 1998, 2008, Kassa et al. 2001). Giardiasis is a common waterborne disease of humans caused by the protozoan, *G. lamblia* (Hamnes et al. 2006, Savioli et al. 2006), also known as *G. intestinalis* and *G. duodenalis*. Nearly 5,000 people in the United States are hospitalized, and millions more worldwide are infected with giardiasis each year (Gardner and Hill 2001). Human infection is caused by ingesting water or feces infected with *Giardia* spp. (Gardner and Hill 2001). The life cycle for all *Giardia* spp. has 2 stages. First, the cyst is shed by an infected host and can persist for a prolonged time in a variety of environments (Figure 1). Second, the trophozoite, which emerges from the cyst under acidic conditions present in the gastrointestinal tract, is the vegetative form that replicates in the small intestine and contributes to the clinical signs of diarrhea and malabsorption (Adam 2001, Thompson 2004; Figure 2). Giardiasis is commonly treated with a class of anti-protozoan drugs known as nitroimidazoles; however, drug resistance can make treatment difficult (Gardner and Hill 2001).

Giardia spp. have been detected in fecal samples of many mammalian species, such as American beavers (*Castor canadensis*), muskrats (*Ondatra zibethica*), cattle (*Bos primigenius*), and

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domestic dogs (*Canis familiaris*; Heitman et al. 2002, Thompson 2004, Applebee et al. 2005). *Giardia* spp. have also been recognized in several avian species (Box 1981, Erlandsen et al. 1990, Upcroft et al. 1997, Filippich et al. 1998, Franssen et al. 2000, Kassa et al. 2001, Kuhn et al. 2002, Majewska et al. 2009). *G. psittaci* causes diarrhea in parakeets (Psittacidae; Panigrahy et al. 1978, Scholtens et al. 1982) and *G. ardeae* has been detected in feces of herons (Ardeidae), egrets (Ardeidae), and ibis (Threskiornithidae; Erlandsen et al. 1990, Kulda and Nohýnková 1995, McRoberts et al. 1996). Attempts to transmit *G. ardeae* to mammals and *G. lamblia* to birds were unsuccessful (Erlandsen et al. 1991). However, viable trophozoites from a clinically ill, wild-caught sulphur-crested cockatoo (*Cacatua galerita*) were propagated in culture and transmitted to laboratory-raised mice (Upcroft et al. 1997). These trophozoites were morphologically indistinguishable from *G. lamblia* and bore no resemblance to *G. psittaci*, suggesting that under certain conditions birds can become biologically competent vectors for *G. lamblia*. Further, detection of *Giardia* cysts in waterfowl feces raises concern that birds may act as mechanical vectors for *Giardia* transmission to susceptible human hosts (Graczyk et al. 2008, Majewska et al. 2009). Our objective was to investigate the presence of *Giardia* spp. in free-ranging, resident Canada geese in parks and greenways in a densely human-populated area of the southeastern United States to improve understanding of the risk of *Giardia* transmission from geese to humans in this region.

Study area

This study was conducted in the Triangle region (Raleigh, Durham, and Chapel Hill) of North Carolina. The Triangle region has many public parks and corporate greenways where resident Canada geese use open surface water and adjacent turfgrass, which also may be used for recreation by people on a daily basis. We focused our study at 9 locations that had geese present for all or most of the year. The average daily number of geese at each site ranged from 10 to 62 birds. The 9 sites were selected for use in a separate study of resident Canada goose behavior and included 3 corporate facilities, 2 parks, a suburban residence, a greenway, a college, and a dairy cow farm (Ayers 2009, Ayers et al. 2010).

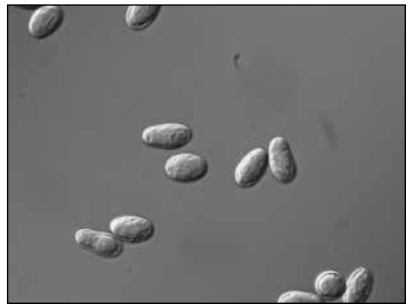

Figure 1. *Giardia lamblia* cysts from a human (*Homo sapiens*) at ×1000 magnification. (*Photo by H. Stibbs*)

Methods

Sample collection

During each summer and fall of 2007 and 2008, we twice collected goose feces from 9 sites (total of 8 collection periods). Each collection was conducted ≤1 week apart, and we collected 4 to 10 fresh fecal samples (<24 hours) at each site. We targeted 10 fresh samples at each collection, but if fewer fresh deposits were available, we collected fewer and did not use older fecal deposits. We used only fresh samples because, while *Giardia* cysts can survive outside of a host for months, desiccation can kill the cysts (Hartman and Kyser 1941). Personnel collecting samples were trained to identify fresh samples based on cylindrical and tubular shape, moist appearance, and presence of geese. Additionally, we selected samples from areas that were previously cleared of droppings within 24 hours as part of a separate study (Ayers et al. 2010). Individual fecal boli (samples) were placed into separate plastic sealable bags, immediately placed on ice, and stored in a freezer at -80° C until testing. Ungar et al. (1984) noted that freezing fecal samples did not adversely affect detection of *Giardia* antigen using enzyme-linked immunosorbent assay (ELISA).

Sample testing

All samples (n = 234) were tested within 2 weeks of collection using the ProSpecT® *Giardia* EZ Microplate Assay (Remel Inc., Lenexa, Kan., USA), an ELISA that uses a monoclonal antibody to detect *Giardia* Specific Antigen (GSA 65, sensitive to 15.6 ng/ml). Each plate

Figure 2. *Giardia simoni* trophozoites from a Norway rat (*Rattus norvegicus*) at ×1000 magnification. (*Photo by H. Stibbs*)

was read visually and spectrophotometrically at 450 nm. Initially, 16 samples were positive spectrophotometrically, but lacked positive visual evidence (yellow color) and were retested. Because the test kit was designed for human fecal samples, a fecal interference test was conducted to ensure that avian feces did not interfere with the testing accuracy. Fifty and 25 µl of the positive control provided with the test kit were added to diluted goose feces, which had previously tested negative, for a total volume of 100 µl. These dilutions were then run in parallel with equal amounts of positive control diluted to 100 µl in buffer. All tests were conducted at the North Carolina State University College of Veterinary Medicine. Also, to supplement results from the ProSpecT® *Giardia* EZ Microplate Assay, 30 additional samples (6 from each of the 5 test sites) were tested for the presence of *Giardia* cysts by immunofluorescence microscopy using AquaGlo™ G/C Comprehensive Kit (Waterborne Inc., New Orleans, La.) that uses an anti-GSA 65 monoclonal antibody.

Results

None of the 234 samples tested positive by enzyme immunoassay. The average optical density of the samples, after subtraction of the negative control value (= 0.0639), was -0.0008 (range -0.0310 to 0.0380), which fell below the 0.05 minimum value of a positive test. All retests were visually and spectrophotometrically negative. For the fecal interference test, after the subtraction of the negative control value, the optical densities for the 50 and 25µl positive controls were 1.816 and 0.898, respectively, compared with 1.651 and 0.811 for positive controls diluted in goose feces, demonstrating no inference by avian feces. Also, none of the samples (n = 30) tested with AquaGlo™ G/C reagent was positive for *Giardia* cysts.

Discussion

The potential for zoonotic transmission of Giardia to humans by waterfowl exists, but the relative risk and importance of transmission remains unclear (Hunter and Thompson 2005, Monis et al. 2009). Initial studies of migratory Canada geese from 9 sites in the Chesapeake Bay area of Maryland indicated that all sites were positive for *Giardia* at an average concentration of 405 cysts/g of fecal material (Graczyk et al. 1998). Similar concentrations of *Giardia* cysts were detected in 18 of 69 fecal samples of hunter-killed wild ducks, primarily mallards (*Anas platyrhynchos*; 13 of 51), along the Rio Grande River near Las Cruces, New Mexico (Kuhn et al. 2002). Further, the Kassa et al. (2001) survey of 16 sites (22 samples) with Canada geese around Toledo, Ohio, detected 2 positive locations (3 positive samples). A study in Poland that included 499 samples from free-ranging, captive, and domestic avian species detected 26 *Giardia* positive birds, twenty-two of which were free-ranging waterfowl (Majewska et al. 2009), including 7 of 32 mallard ducks, 10 of 34 greyleg geese (*Anser anser*), 1 of 72 common mergansers (*Mergus merganser*) and 4 of 34 mute swans (*Cygnus olor*). The results of these studies indicated that migratory waterfowl may be at greater risk of exposure to *Giardia* than resident populations, and the risk of exposure varies by geographic location.

To detect *Giardia*, we selected an enzyme immunoassay for this survey study because of ease of use, relatively inexpensive cost, and demonstration of high specificity and sensitivity in different susceptible hosts (Johnston et al. 2003, Mekaru et al. 2007, Rimhannen-Finne et al. 2007). The ProSpect® test kit used in this study was developed to recognize a 65 kD glycoprotein present on trophozoites and cysts of *Giardia lamblia* (Rosoff and Stibbs 1986). Subsequently, Stibbs (1989) demonstrated the anti-GSA 65 monoclonal antibody cross-reacted with cysts of *G. muris* and reacted with cysts of *G. microti* (a.k.a. *G. ondatrae*) from naturally infected muskrats (*Ondatra zibethicus*). Additionally, antigen-capture ELISA has been used to detect cell-free antigens in muskrat

feces (H. Stibbs, co-owner of Waterborne Inc., New Orleans, La., unpublished data). Kassa et al. (2001) employed a similar Prospect® test kit that used the anti-GSA 65 monoclonal antibody to positively identify *Giardia* in the feces of Canada geese within a 32-km radius of Toledo, Ohio.

The North Carolina Triangle, comprised of Raleigh, Durham, and Chapel Hill is relatively urban, and the opportunity for humans to encounter feces of Canada geese near surface waters in parks and greenways is great. Additional research should be conducted to determine the risk of exposure to *Giardia* through feces of Canada geese in similar parts of the country. Although commercially available enzyme immunoassays are a relatively inexpensive and quick way to detect *Giardia* in avian feces, research is needed to determine which genetic assemblages of *Giardia* occur in waterfowl and whether birds actually contribute to human infections. In urban settings, monitoring resident Canada geese and other wildlife for *Giardia lamblia* assemblages A and B might indicate areas that compromise ecosystem and human health. Our results indicate that deposition of *Giardia* from Canada geese in areas of human recreation and use in the Triangle region of North Carolina was unlikely during the time period of this study. However, periodic testing of goose feces for *Giardia* is warranted.

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