# Final Report – SG 124-99
(November 30, 2000)

## *"Surveillance of Waterfowl Feces for Presence of Microbes Pathogenic to Humans"*

Michael Bisesi, PhD (Principal Investigator)
Professor and Chair, Department of Public Health
Medical College of Ohio
Toledo, OH 43614

Brian Harrington, PhD (Co-Investigator)
Adjunct Professor, Department of Public Health
Medical College of Ohio
Toledo, OH 43614

Hailu Kassa, PhD (Co-Investigator)
Adjunct Assistant Professor, Department of Public Health
Medical College of Ohio
Toledo, OH 43614

## INTRODUCTION

The Medical College of Ohio (MCO) is an academic health center that engages in education, research, and service. One of the major goals of the Department of Public Health at MCO is to conduct research that contributes to the prevention and/or minimization of human illness via assessment and remediation of potentially adverse environmental and occupational exposures to chemical, physical, and biological agents. Accordingly, the primary objective for this pilot study was to conduct sampling and analysis of a potential nonpoint source of pathogenic microbial soil, shoreline, and water contamination. This study focused on identifying sites contaminated with fecal droppings of giant Canada geese and evaluating collected specimens for *Cryptosporidium*, *Giardia*, and *Campylobacter*, using sensitive monoclonal enzyme immuno assay (EIA) methods and microscopy. The rationale for collection and subsequent analyses of geese feces for

*Cryptospridium spp., Giardia lamblia, and Campylobacter spp.* was based on concern for potential contamination of waterways and adjacent areas with these major pathogenic microbes known to cause serious human gastrointestinal infections. Indeed, these organisms are three of the most common causative agents of gastroenteritis. Despite scientific documentation of the presence of these pathogenic microbes in waterfowl feces (Luechtefeld et al. 1980; Graczyk et al. 1998; Eichorst et al. 1999) and surface waters (Carter et al. 1987) there is apparently some debate locally regarding this issue based on a relatively recent nonscientific report (Tressler, 1999).

Giant Canada geese (*Branta canadensis maxima*), which are nesting locally in Northwest Ohio and other parts of the state, are commonly perceived as a public nuisance when they inhabit urban areas. This is a result of large numbers of Canada geese littering the ground with their feces in areas of human recreational and occupational activity. Indeed, the feces of giant Canada geese litter both grass and pavement areas adjacent to bodies of water at many recreational and occupational sites in the Toledo area, throughout Northwest Ohio, and elsewhere.

Between February and March, mating pairs of giant Canada geese disperse to their breeding territories to lay eggs and raise young. Flocks of giant Canada geese seen during the months of April-July are non-breeding groups. After breeding, and beginning in late August, family units move between their roosting and feeding habitats. These are often located in urban sites that contain ponds and grassy areas. During the winter, giant Canada geese remain sedentary, moving only as far south as southern Ohio or Kentucky in extremely cold conditions (personal communication, Mark Shieldcastle, Wildlife Biologist, Ohio Division of Wildlife).

During the non-breeding season, flocks of giant Canada geese can be seen on golf courses and in public parks, as well as around hospitals, nursing homes, wastewater treatment plants, grain storage elevators, cemeteries, and in many other workplaces containing bodies of water and grassy areas. The contact between people and giant Canada geese typically increases at this time, and there is great concern that these birds pose a potential health threat to people exposed to their fecal droppings. Indeed, since fecal droppings of giant Canada geese are dense in many sites, recreational and occupational exposures to pathogenic microorganisms, such as *Cryptosporidium*, is very plausible.

## METHODS AND MATERIALS

Fecal droppings of giant Canada geese (*B. canadensis maxima*) were collected at random from 21 different sites in Toledo, Ohio and surrounding Northwest Ohio communities during spring and fall 1999 and summer through fall 2000. The outdoor sites included public parks, golf courses, an industrial site, cemeteries, and health care facilities where bodies of water, both large and small, were located. Thirty-seven composite samples were collected from the 21 sites during the study period. Each composite sample consisted of 12 freshly deposited feces placed into sealable sandwich bags. The feces in each bag were mixed and compressed manually into one composite sample. They were then divided into two aliquots, one of which was archived at -20 °C. The other was analyzed for *Cryptosporidium*, *Giardia*, and *Campylobacter*.

Fecal droppings were analyzed for *Cryptosporidium, Giardia*, and *Campylobacter*, using ProSpect Microplate Assay kits (Alexon-Trend, Inc, 1400 Unity

St. NW, Ramsey, MN 55303). The method of detection was a solid phase enzyme immuno assay (EIA) technique, using monoclonal antibodies specific for *Cryptosporidium* (CSA), *Giardia* (GSA 65), and *Campylobacter* antigens. These colorimetric kits have been widely accepted for clinical use due to the high degree of specificity and sensitivity associated with the monoclonal antibodies and the methodology. The analytical sensitivities for *Cryptosporidium, Giardia*, and *Campylobacter* were approximately 20 ng/ml, 3.9 ng/ml, and 2.81 ng/ml respectively. Cross reactivity studies have shown that *Campylobacter jejuni* and *Campylobacter coli* share the *Campylobacte*r specific antigen. All three assays were read and interpreted visually according to the parameters and color charts supplied with the assay kits. Although there was no biased interest in some sites being positive and others negative, for quality control purposes, the laboratory technician was unaware of the specific sources of the specimens. In addition, both positive and negative controls were run with each set of analyzed specimens.

## RESULTS

Fecal droppings of Canada geese were concentrated around bodies of water and surrounding grassy areas. The amount of droppings varied from area to area, averaging 10 to 12 droppings per square meter. Of the 37 composite samples from 21 distinct sites, 14 were completely negative while 23 were positive for one or more of the pathogens (see Table 1). Of the 23 positive samples, 21 (91.3%) were positive for *Cryptosporidium*, eight (34.8%) were positive for *Campylobacter*, and four (17.4%) were positive for *Giardia*. Of the 21 sites, four were completely negative while 17 were positive for at least one, but not all three, of the pathogens. Of the 17 sites from which composite samples tested positive for at least one of the three pathogens evaluated, 16 (94.1%) were positive for *Cryptosporidium,* eight (47.1%) were positive for *Campylobacter*, and three (17.6%) were positive for *Giardia*. Sites positive for *Giardia* were not positive for *Campylobacter* and vice versa; however, the two *Giardia* positive sites and six out of the eight *Campylobacter* positive sites were also positive for *Cryptosporidium*. Four out of five golf courses were positive for *Cryptosporidium*, of which two were also positive for *Campylobacter.* Composite samples collected at Collins Park wastewater treatment plant and Maumee Bay State Park were negative for all three pathogens evaluated, however, the sample from Crane Creek State Park tested positive for *Cryptosporidium*.

**Table 1.** Giant Canada geese (*Branta canadensis maxima*) sites with fecal droppings positive (+) or negative (-) for *Cryptosporidium*, *Giardia*, and *Campylobacter* as determined by monoclonal EIA method.

| Site | Collection Date | Crypto | Campy | Gia |
|---|---|---|---|---|
| Collins Park Wastewater Treatment | 9/19/99 | - | - | - |
| | 10/4/99 | - | - | - |
| Hecklinger pond | 5/8/99 | + | - | - |
| | 9/19/99 | - | - | - |
| Toledo Memorial Cemetery (2 areas) | 9/19/99 | + + | - - | + + |
| Flower Hospital Pond | 9/19/99 | + | - | + |
| Arlington/Detroit Ave Pond | 5/18/99 | + | + | - |
| | 9/19/99 | + | - | - |
| Detwiler Golf Course | 5/18/99 | + | - | - |
| | 9/19/99 | - | - | - |
| Side Cut Park | 9/21/99 | + | - | - |
| Maple St. Boat Launch, Perrysburg | 9/23/99 | + | + | - |
| Belmont Golf Course | 3/26/99 | - | - | - |
| | 10/3/99 | + | + | - |
| Grain Elevator By River On I 75 | 10/3/99 | - | + | - |
| Levis Development Park, Perrysburg | 10/3/99 | + | + | - |
| Owens Technical College, Rossford | 10/3/99 | + | + | - |
| Stone Oak Golf Course | 10/4/99 | - | - | - |
| Toledo Botanical Garden | 10/5/99 | + | - | - |
| Brandywine Golf Course | 10/20/99 | + | + | - |
| Automotive Parts Plant, Rossford | 11/22/99 | - | - | - |

| Site | Collection Date | Crypto | Campy | Gia |
|---|---|---|---|---|
| Levis Development Park, Perrysburg | 8/26/00 | - | - | - |
| Hecklinger Pond | 8/26/00 | + | - | - |
| UT Scott Park | 8/26/00 | - | - | - |
| Detwiler Golf Course | 8/26/00 | - | - | - |
| Flower Hospital Pond | 6/8/00 | + | - | - |
| Stone Oak C.C. Golf Course | 6/25/00 | - | - | - |
| Crane Creek State Park | 6/25/00 | + | - | - |
| Maumee Bay State Park | 6/25/00 | - | - | - |
| BGSU Golf Course | 6/26/00 | + | - | - |
| Davis Besse | 6/25/00 | - | + | - |
| Arlington/Detroit Ave. Pond | 7/24/00 | + | - | - |
| Woodlawn Cemetary | 7/25/00 | + | - | - |
| Owens Tech | 8/20/00 | - | - | - |
| Automotive Parts, Rossford | 8/20/00 | + | - | + |
| Toledo Memorial Cemetary | 8/20/00 | - | - | - |

**Note:** Crypto = *Cryptosporidium*, Campy = *Campylobacter*, Gia = *Giardia*

## DISCUSSION

Since family units of Canada geese move between different roosting and feeding sites and mix with other family units, sites that are negative on one occasion may become positive on another occasion. Due to the high density of fecal droppings at positive sites, humans with recreational or occupational exposure to the feces of giant Canada geese or contaminated surfaces face the risk of infection with one or more of these pathogens.

It was reported that as few as 30 *Cryptosporidium* oocysts can cause illness in a healthy adult (DuPont et al. 1995), but the concentration of *Cryptosporidium* oocysts in individual fecal droppings of Canada geese was considerably higher than the minimum infective dose (Graczyk et al. 1998). Therefore, given the low dosage level required to cause infection, and considering that more sites were positive for *Cryptosporidium* than for *Giardia* or *Campylobacter*, this study suggests that *Cryptosporidium* poses the highest risk of infection to humans who are recreationally or occupationally exposed to the feces of giant Canada geese.

Ground maintenance workers and gardeners on golf courses, in public parks, and in other areas frequented by giant Canada geese are possibly more at risk of infection to *Cryptosporidium* than any recreational users. This is because they have a higher chance of contacting fecal droppings or environmental surfaces contaminated with the feces of giant Canada geese. These workers are at considerable risk for contaminating their hands, clothes, and shoes with *Cryptosporidium* oocysts and, to a lesser extent, with *Campylobacter* and *Giardia* cysts. Therefore, hand-to-mouth contact is the primary mode of transmission of these pathogens to workers.

Since oocysts of *Cryptosporidium* remain viable and infective below freezing temperature (Fayer and Nerad, 1996), the potential risk of occupational exposure to *Cryptosporidium* may not diminish due to seasonal variations in temperature. In addition to the fecal-oral route, workers may be exposed *to Campylobacter*, *Cryptosporidium*, and *Giardia* via contact with contaminated environmental surfaces.

Although, the wastewater treatment plant was negative in this study, wastewater treatment plant workers are still at risk for exposure to *Cryptosporidium* oocysts and *Giardia* cysts, since fecal droppings of Canada geese can contaminate watersheds (LeChavallier et al. 1991; Ong et al. 1996). In fact, *Cryptosporidium* and *Giardia* could have been among the pathogens responsible for the high incidence of gastroenteritis in wastewater treatment plant workers reported in one study (Khuder et al. 1998). In addition to accidental contact with infected fecal droppings of Canada geese or with environmental surfaces contaminated with their feces, inadequately treated drinking water could be a source of exposure to *Cryptosporidium* and *Giardia* . One study reported finding *Cryptosporidium* oocysts and *Giardia* cysts in potable water contaminated with the feces of Canada geese (Hsu et al. 1999). It is possible that the concentration of bleach used to disinfectant the potable water might not have been strong enough to kill the *Cryptosporidium* oocysts and *Giardia* cysts. The normal concentration of bleach used to disinfect water was found to be ineffective against *Cryptosporidium parvum* (Fayer, 1995).

In spite of the occurrence of *Cryptosporidium* in many animal species, the reported incidence of cryptosporidiosis in humans is very low. In the Toledo area, there was one reported case of cryptosporidiosis in 1998, and there were none in 1999 (Unpublished report, Ohio Department of Health, Disease Surveillance Section). Lack of routine examination of stools by local laboratories could be one reason for the low incidence levels of cryptosporidiosis reported in Ohio and elsewhere. It was reported that the number of stool samples that tested positive for *Cryptosporidium* increased when laboratories routinely used a combination of detection methods (Roberts et al. 1996). Indeed, based on the results from our study, we recommend that physicians in Northwest Ohio routinely request *Cryptosporidium* testing of the stools of patients working in outdoor areas or patients presenting with applicable symptoms exposed to areas occupied by Canada geese. In relation, clinical laboratories must consider using the most sensitive detection method or methods available.

The results generated from our pilot study are very interesting and important relative to public health. We have generated data that refutes opinion originally stated in an article that appeared in the *Toledo Blade* during March 1999 (Tressler, 1999). In relation, a manuscript related to this research was accepted recently for publication in the *Journal of Applied Occupational and Environmental Hygiene*. This project addressed the Ohio Lake Erie Commission's priority category "Pollution Prevention/Reduction Implementation Projects". Plans are underway by us to expand this pilot-scale research into a larger-scale study to further investigate this topic. We want to continue research that will contribute to the protection of humans recreationally and occupationally exposed to shoreline areas contaminated with biological pathogens. Data from the research will contribute to activities that promote/evaluate development and implementation of new and innovative practices to reduce nonpoint source pollution and/or activities that may cause exposure to these contaminated areas.

## REFERENCES


Carter, A.M., Pacha, R.E., Clark, G.W., and Williams, E.A. (1987). Seasonal Occurrence of *Campylobacter* spp. in Surface Waters and Their Correlation with Standard indicator Bacteria. Appl. Env. Micro. 53:3, 523-526.

DuPont, H.L.; Chappell, C.L.; Sterling, C.R.; et al. (1995). The Infectivity of *Cryptosporidium parvum* in Healthy Volunteers. N. Engl. J. Med. 332(13): 855-859.

Eichorst, S., Pfeifer, A., Filer, S., Markos, M., and Tischler, M.L. (1999). Presence of Parasitic Protozoa from Fecal Samples of Wild Populations of Resident Canada Geese. Abstracts in Env. Genl. Appl. Micro. ASM Session 69/Q-96 May 31, 1999.

Fayer, R. (1995). The Effect of Sodium Hypochlorite Exposure on Infectivity of *Cryptosporidium parvum* Oocysts for Neonetal BLAB/c Mice. Appl. Environ. Microbiol. 61:844-846.

Fayer, R. and Nerad, T. (1996). Effect of Low Temperature on Viability of *Cryptosporidium parvum* Oocysts. Appl. Environ. Microbiol. 62: 1431-1433.

Graczyk, T.K., Fayer, R., Trout, J.M., Lewis, E.J., Farley, C.A., Sulaiman, I., and Lal, A.A. (1998). *Giardia* sp. Cysts and Infectious *Cryptosporidium parvum* Oocysts in the Feces of Migratory Canada Geese. Appl. Env. Micro. 64:7, 2736-2738.

Hsu, B.M.; Huang, C.; Jiang, G.Y.; et al.: The prevalence of *Giardia* and *Cryptosporidium* in Taiwan Water Supplies. J. Toxicol. Environ. Health. 57(3): 149-160 (1999).

Khuder, S.A.; Arthur, T.; Bisesi, M.S. et al. (1998). Prevalence of Infectious Diseases and Associated Symptoms in Wastewater Treatment Workers. Am. J. Ind. Med. 33(6): 571-587.

LeChevallier, M.W.; Norton, W.D.; Lee, R.G. (1991). Occurrence of *Giardia* and *Cryptosporidium* sp. in Surface Water Supplies. Appl. Environ. Microbiol. 57(9): 2610-2616.

Luechtefeld, N.A.W., Blaser, M.J., Barth Reller, L., and Wen-Len L. Wang (1980). Isolation of *Campylobacter* fetus subsp. *jejuni* from Migratory Waterfowl. J. Clin. Micro., 12:3, 406-408.

Ong, C.; Moorehead, W.; Ross, A.; et al. (1996) Studies of *Giardia* sp. and *Cryptosporidium* spp. in Two Adjacent Watersheds. Appl. Environ. Microbiol. 62(8): 2798-2805.

Roberts C.L.; Morin, C.; Addiss, D.G.; et al. (1996): Factors Influencing *Cryptosporidium* Testing in Connecticut. J. Clin. Microbiol. 34(9): 2292-2293.

Tressler, M. (1999). Many Down on Canada Geese. Toledo Blade, March 15, 1999.

## ACKNOWLEDGMENTS

Much thanks for the funding provided by the small grant from the Lake Erie Protection Fund. We also wish to thank David Welch, Sanitarian, Toledo-Lucas County Department of Health for assisting in the collection of fecal samples and Mark Shieldcastle, Wildlife Biologist, Ohio Division Of Wildlife, for his valuable information regarding the breeding and migration patterns of giant Canada geese in Ohio.