A SURVEY TO ESTIMATE THE PREVALENCE OF *Salmonella* sp., *Shigella* sp., *Yersinia* sp. BACTERIA AND *Cryptosporidia* sp., *Giardia* sp. PROTOZOA IN RESIDENT CANADA GEESE (*Branta canadensis*) IN NEW JERSEY.

ABSTRACT:

Five hundred flightless Canada geese were captured at 16 locations in New Jersey between 6/25/99 and 7/9/99. No *Salmonella* sp., *Shigella* sp., or *Yersinia* sp. bacteria where isolated from cloacal swab cultures which suggests Canada geese do not pose a significant source of environmental contamination and transport of these pathogens in New Jersey. *Cryptosporidia* sp. and *Giardia* sp. were relatively common occurring in 10% and 15% of the Canada geese, respectively. They also occurred with approximately twice the frequency in juveniles as in adult birds. This was thought to be due to acquired resistance. These protozoa are widespread in New Jersey with Cryptosporidium and Giardia occuring at 88% of the sites. Due to sample limitations no mouse bioassay or genotyping through PCR was performed and the zoonotic nature of these organisms is not known. Future research should focus on identifying the genotypes of the protozoa as well as sources in the habitat at selected NJ locations.

BACKGROUND AND JUSTIFICATION:

The resident Canada goose (*Branta canadensis*) population in New Jersey has doubled between 1989 and 1996 (Castelli, unpublished data). These non-migratory geese pose nuisance problems at swimming ponds, golf courses, parks and industrial park lawns throughout New Jersey. It is frequently alleged that the goose feces, which are part of the nuisance issue, also pose a health hazard. No human disease outbreaks have been directly linked to exposure to goose feces and there is limited information on the frequency of human pathogens in goose feces. *Salmonella* sp. bacteria were cultured from cloacal swabs of 8 of 575 flightless Canada geese from park settings at 15 locations throughout New Jersey during June and July 1994 and June, July and August 1995 (Bigus, 1996; Roscoe, 1996). *Shigella* sp. bacteria were also cultured from cloacal swabs from 3 of these Canada geese. *Shigella* sp. were cultured from cloacal swabs from 2 of 4 snow geese (*Chen caerulescens*). These potential human pathogens were associated with geese at sites of known human sewage contamination with the single exception of a *Salmonella* sp. from the Forsythe National Wildlife Refuge. *Yersinia* sp. bacteria were isolated from a Canada goose from Palatine Lake in Salem county during the Bigus survey and this has been listed amoung the potential human pathogens harbored by wild birds (Brittingham et al., 1988).

As part of a study of *Cryptosporidium parvum* contamination of oysters in the Chesapeake Bay drainage fecal samples from Canada geese were collected near

cattle farms. They were found to contain *Cryptosporidium parvum* oocysts (Graczyk, 1998). Cryptosporidia in unfiltered water has caused several outbreaks of diarrheal disease in humans in the United States. Cryptosporidiosis is listed among the Center
for Disease Control and Prevention's emerging pathogens. At least one outbreak of diarrheal disease from this pathogen has been documented in people swimming in a park pond in New Jersey. Another enteric protozoan pathogenic to man includes *Giardia lamblia*. Giardia have been described from over 40 species of animal hosts (Kulda and Nohynkova, 1978).

The purpose of this study was to determine if *Salmonella*, *Shigella* and *Yersinia* sp. bacteria and *Cryptosporidium* sp., *Giardia* sp. cysts are present in flightless Canada geese from 16 selected sites in New Jersey. If detected a secondary goal was to estimate the prevalence of these potential human pathogens in the resident goose population and their habitat.

MATERIALS AND METHODS:

Sample considerations

Previous Canada goose banding operations of the New Jersey Division of Fish and Wildlife during the months of June and July have resulted in captures of 5,000 birds. Estimates of disease prevalence in fish populations of that size require a sample size of 438 to be 99% confident that at least one diseased animal will be detected if the disease is present in 1% of the population (Simon and Schill, 1984). By using "the rule of three" (Hanley and Lippman-Hand, 1983) n=3/P where P is the estimated prevalence a sample size of 300 (n) is required to be 95% confident that at least one diseased animal will be detected if the disease is present in 1% of the population. A sample size of 500 deer were used from a population of 100,000 to be 99% confident that TB or CWD would be detected if it occurred in 1-3% of the population in New Jersey based on Epistat estimates performed by NJ USDA VS. The sample size of 454 (n) required to detect one infected individual in a population with a 1% prevalence of infection (P) at a confidence level of 99% was determined by solving n=log(1-C)/ log(1-P) (DiGiacomo and Koepsell, 1986).

A sample size of 500 Canada geese was considered adequate (95-99% confidence) to detect the various pathogens in this survey if they were present in at least 1% of the birds. An attempt was made to include half (250) juveniles and half adults (at least 1 year old) in the sample from each site. The juveniles would presumably reflect local recent exposure and not a chronic infection which might be possible in adults. Banded adults were selected in order to have historic data on the birds movements. Sixteen sample sites were selected based on the likelihood at least 15 juveniles and 15 adults could be captured per site, to insure a wide geographic

representation of the state resident goose population, to include sites of previous bacterial pathogen positivity and represent areas (Monmouth County) where epidemilogic data suggests possible human giardiasis from Canada goose fecal contamination. Four of these sites will be utilized by the USGS in their study on screening for potential human pathogens in fecal material deposited by resident Canada geese on areas of public utility (unpublished USGS, 1999 proposed study plan).

Goose capture methods and data collection

Flightless birds were herded toward nets which were mounted on rectangular aluminum frames. These nets were carried toward the group of birds until the birds were completely surrounded. The net frames were bound together and geese were hand captured by personnel of the New Jersey Division of Fish and Wildlife. The birds were aged, sexed and banded with US Fish and Wildlife Service leg bands. Band numbers from previously captured adults were recorded.

Bacteriologic sampling and analysis

Salmonella and *Shigella*

Sterile swabs after insertion into the cloaca and rectum were rotated removed and placed in a buffered glycerol saline transport medium. Samples were placed in a cooler on a layer of paper towels over "Blue Ice" and delivered to NJ Department of Agriculture, Division of Animal Health Laboratory within 48 hours of collection. A MacConkey's plate was streaked from the transport media and incubated at 30^0C for 48 hours. A cefsulodin irgasan novobiocin (CIN) plate was streaked from the transport media and incubated at 30^0C. One milliliter of the transport media was inoculated into tetrathionate broth and phosphate buffered saline pH 7.0 and a gram negative broth. The tetrathionate broth was incubated at 37^0C for 24 hours and plated to SS, HE and brilliant green (BG). The gram negative broth was incubated for six hours and plated to SS, HE and BG. The plates are placed in a 37^0C incubator and read at 24 hours. A delayed secondary enrichment involves holding the tetrathionate broth at room temperature for 1 week. One milliliter was transferred to a new tetrathionate broth, incubated for 24 hours and plated on SS, HE and BG again. Suspect colonies were to be transferred to TSI, LIA and TSA slants and incubated at 37^0C for 24 hours. The positives were to be inoculated in the API 20E. True positive isolates were to be submitted to the National Veterinary Services Laboratory for serotyping.

Yersinia

One milliliter of the transport media containing the cloacal swab was inoculated into PBS and held in the refrigerator for one week. Every week for 3 weeks the sample was plated to CIN and incubated at 30^0C and plated to MacConkey's and incubated at 37^0C for 48 hours. Suspect colonies were to be inoculated to TSI, LIA, and TSA slants. Suspect colonies were to be transferred to API 20E and incubated at 30^0C for identification.

Protozoan sampling and analysis

Loops of feces collected from the cloaca and lower intestine were used to inoculate the well in a glass slide in the field. The air dry samples or formalin fixed samples were subjected to the direct immunofluorescent antibody test for *Cryptosporidium* sp. and *Giardia* sp..(Meridian Diagnostics, Inc., Cincinnati, OH 45244) The detection reagent is FITC labeled anti-Cryptosporidium cell wall and anti-Giardia cell wall monoclonal antibodies (Garcia et. al., 1987).

Twenty liter water samples collected at each site were filtered through gauze, then through a 3.0 μm Millipore membrane (Millipore SSWp,47 mm cat# SSP04700). The collecting side was scraped, flushed and washed three times in 300ml distilled water. After 10 minutes to allow settling of fibers and debris the water was filtered through Nucleopore Polycarbonate membrane 0.8μm which was supported by another membrane(Nucleopore polycarbonate 25 mm, 0.8μm. Costar Cat# 110659). This membrane was placed (shiny side up) on a microscope slide stained with one drop of Fluorescence conjugated monoclonal anti-*Cryptosporidium* oocyst/*Giardia* cyst cell wall, counterstained (Meridian Diagnostics, Inc., Cincinnati, OH 45244) and incubated in a dark container in a humidified chamber for 30 minutes at 37^0 C. The membrane was then washed 3 times with 1X buffer. The excess buffer was absorbed by a clean paper towel. A drop of mounting medium was placed on the membrane and the coverslip applied. The slides were examine under a UV microscope for *Giardia* sp. cysts and *Cryptosporidium* sp. oocysts.

Data and statistical analyses

Data on the protozoan survey and data from bacteriology will be transformed into prevalence figures by dividing the number of birds sampled by the number positive for a given pathogen and converted to percent. Statistical comparisons of prevalence amoung age groups and locations will be analyzed using the Chi-square test or Fisher's exact test in the event that some cells have expected counts of <5 (SAS,

1987). Comparisons of *Cryptosporidium* sp. and *Giardia* sp. in the water and prevalence in geese resident at the site will be made in a qualitative manner. This study may be supplemented with findings from the USGS National Wildlife Health Center survey for human pathogens in goose feces from 4 New Jersey ground collection sites.

RESULTS

Five hundred flightless Canada geese were captured at 16 locations in New Jersey between 6/25/99 and 7/9/99. One hundred twenty-five were young of the year males (J-M), 108 were young of the year females (J-F), 137 were adult males (A-M) and 130 were adult females (A-F).

No *Salmonella* sp.*, Shigella* sp. or *Yersinia* sp. were isolated from any of the 500 Canada goose cloacal swab cultures.

Cryptosporidium sp. was found in birds from all sites except four (Cooper River, Palatine Lake, Mercer County Golf Course, Shepard Lake) (Table 1). Forty-nine (10 %) of the 500 geese had *Cryptosporidium* sp. in cloacal loop samples. Thirty-two (65 %) of these 49 birds were young of the year (17 males, 15 females) versus 17 (35 %) which were adults (5 males, 12 females). This difference in prevalence between juveniles and adults was highly significant ($\chi^2 = 7.639$, 1df, P = 0.006). There was no significant difference with respect to sex ($\chi^2 = 1.226$, 1df, P = 0.268). *Cryptosporidium* sp. cysts were detected in 7 (47 %) water samples collected from 15 sites.

Giardia sp. was found in birds from all sites except two (Mercer County Golf Course, VanSaun)(Table 1). Seventy-five (15%) of the 500 geese had *Giardia* sp. in cloacal loop samples. Fifty-two (69%) of these 75 birds were young of the year (27 males, 25 females) versus 23 (31%) which were adults (11 males, 12 females). This difference in prevalence between juveniles and adults was highly significant ($\chi^2 = 18.325$, 1df, P = 0.001). There was no significant difference with respect to sex ($\chi^2 = 0.106$, 1df, P = 0.744). *Giardia* sp. cysts were detected in only 2 (13 %) of the 15 water samples.

Nine (8%) of the 115 protozoan positive geese were positive for both C*ryptosporidium* sp. and *Giardia* sp.. Seven (78%) of those positive for both protozoa were young of the year (4 males, 3 females) versus 2 (22%) which were adults (0 males, 2 females). The difference in prevalence of dual "infections" between those adults and juveniles which had protozoa in the sample was not significant (P = 0.716, Fisher's Exact Test, 2-Tail). Similarly there was no difference between sexes (P = 1.0, Fisher's

Exact Test, 2-Tail). Both protozoans were detected in water samples from the Spruce Run Reservoir and the Raritan River at Johnsons Park.

Microporidia sp. were found in a water sample from the Rahway River Park pond.

DISCUSSION:

The absence of isolates of *Salmonella* sp., *Shigella* sp. and *Yersinia* sp. suggests these organisms if present in the New Jersey resident geese are in less than 1% of the population at a confidence level of 99% (DiGiacomo and Koepsell, 1986). A previous survey of resident Canada geese at 15 New Jersey locations during the summer of 1994 (Bigus 1996) suggests a *Salmonella* sp. prevalence of 1.4% (8/575). He also found *Shigella* sp. in cloacal swabs from 3 geese (0.5%) and *Yersinia* sp. in 1 goose (>0.5%). Confirmations of these isolates by Bigus through reculturing were not successful which may have been attributable to the cultures dying out or being overgrown by other species. Extensive sampling of Canada geese and tundra swans (*Cygnus columbianus columbianus*) for Salmonella and Shigella organisms were unsuccessful and lead Damare et al., (1979) to conclude that healthy waterfowl in the wild, away from polluted environments, do not harbor these enteric pathogens. These bacteria appear to be rare in the resident Canada goose populations and pose no important source of these organisms for water contamination or human/animal exposure in New Jersey.

Cryptosporidia and Giardia were relatively common occurring in 10% and 15% of resident Canada geese sampled in New Jersey. Graczyk and co-workers (1998) found Cryptosporidium and Giardia in the feces collected from the ground at 78% and 100% for nine sites in Maryland, respectively. Our survey of resident geese and water samples also found these organisms to be widespread (88% of the sites). Cryptosporidia were found in at least one Canada goose from 75% (12) of the sites and Giardia were found in at least one Canada goose from 88% (14) of the sites. At least one of these protozoa was found in geese from all but one site (Mercer County Golf Course). Two Cryptosporidia contaminated sites (Mercer County Golf Course, Palatine Lake) would have been missed if water samples had not been tested. While resident Canada geese were the best single indicator of site contamination with Cryptosporidia and Giardia, the single sample method employed in this study would fail to detect periodic or irregular oocyst/cyst shedding events. Graczyk found the larger the Canada goose fecal sample the greater the number of recovered oocysts. Six percent fewer Cryptosporidium postive tests may be expected in testing unconcentrated fecal samples such as those in this study versus concentrated fecal samples (Meridian Diagnostics, Inc., 1997). No similar change in detection was observed using Giardia. It is reasonable to conclude the prevalence figures derived in this survey constitute a minimum figure.The relatively frequent negative result of the water sample

tests may reflect a dilution effect. Future surveys for the two protozoans in this study should include both resident geese and water sampling in the experimental design.

Cryptosporidia and Giardia were twice as prevalent in juvenile geese as in adults. This suggests Canada geese may acquire immunity from exposure to these two enteric protozoan parasites. Giardia infections in parakeets is most commonly diagnosed in young birds and resolves as the birds reach maturity. When these uninfected or recovered birds were placed in proximity to infected birds active shedding was induced indicating a lack of host immunity (Scholtens et. al., 1982).

A Giardia species from a wild sulfur-crested cockatoo (*Cacatus galerita*) was maintained in vitro and chronically infected mice (Upcroft et. al., 1997). Earlier attempts by Erlandsen and co-workers (1991) to transmit avian Giardia species to mammals and visa versa were not successful. While we do not know the zoonotic nature of the Giardia cysts collected from the resident Canada geese we do know the test kits commonly employed in testing drinking water gave positive results for the Giardia of geese as was similarly noted by Graczyk and co-workers (1998). Therefore, the contamination of surface waters with goose feces could potentially confound current monitoring methods for human pathogenic *Giardia sp.*.

Cryptosporidium parvum, the human pathogen, is unable to establish intestinal infection in Canada geese (Graczyk et. al., 1996, 1997, 1998). Mouse bioassay and molecular genotyping (PCR) employed in their studies demonstrated the oocysts recovered from Canada goose feces was the zoonotic genotype of *C. parvum.* From this they concluded the geese were functioning as a transport host and the *C. parvum* cysts were being acquired from the habitat. They implicated cattle manure containing undigested corn as a potential source and found an identical genotype of *C. parvum* in oysters at the study site. While *Cryptosporidium sp.* recovered from the cloaca of the resident geese in the New Jersey study gave a positive response to the fluorescent labelled monoclonal antibody there is the possibility the species were not zoonotic (*C. parvum*) due to cross-reactivity by *C. melegridis* and other non-zoonotic species. The current study protocol consumed the entire sample so no mouse bioassay or molecular genotyping was possible. It is not clear if acquired resistance to *C. parvum* would be functioning in a transport host which was not actively infected. Future research should focus on collection of cloacal specimens from geese at a few sites of prevalent infection with mouse bioassay and PCR testing to confirm zoonotic *C. parvum.*

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PROJECT TIME FRAME:

Field work from June 1, 1999 - August 15, 1999
Laboratory analysis completed by September 15, 1999
Publishable reports completed by December 31, 1999

PROJECT COSTS:

Project oversight, goose capture, report preparation; NJ Division, Fish and Wildlife

Name	Project Title	Pay Rate*	Hours	Cost
Douglas E. Roscoe, Ph.D.	State Project Officer	$57.87	70	$4051
Melissa Craddock	Seasonal Technician	$10.90	600	$6541
subtotal				**$10,592**

* fringe and indirect included

Project bacteriology; NJ Department of Agriculture, Division of Animal Health

500 samples cultured for Salmonella, Shigella, Yersinia and speciation of same.

subtotal **$5,000**

PROJECT TOTAL COST **$15,592**

PREPARED BY:_____ **DATE:** December 30, 1999
Douglas E. Roscoe, Ph.D., Project Officer NJDFW Revised 2/3/00

APPROVED BY:_____ **DATE:**_____
Dave Chanda, Assistant Director, NJDFW

APPROVED BY:_____ **DATE:**_____
Fred Snyder, Federal Aid Coordinator, NJDFW

APPROVED BY:_____ **DATE:**_____
George Haas, Project Officer USFWS

TABLE 1. Prevalence of *Cryptosporidium* sp. and *Giardia* sp. in resident New Jersey Canada geese.

LOCATION/DATE	Prevalence of **Cryptosporidium** positive geese by age-sex class				Prevalence of **Giardia** positive geese by age-sex class				Prevalence of **Crypto** and **Giardia** positive geese by age-sex class				**WATER**	
Latitude/Longitude	A-M	A-F	J-M	J-F	A-M	A-F	J-M	J-F	A-M	A-F	J-M	J-F	Crypto.	Giardia
Cooper River Pk/ 6/25 Camden County N 39^0 55.476' W 75^0 7.987'	0/7	0/8	0/8	0/7	0/7	**1/8**	**2/8**	0/7	0/7	0/8	0/8	0/7	0	0
Palatine Lake/ 6/28 Salem County N 39^0 32.390' W 75^0 10.200'	0/7	0/8	0/5	0/10	0/7	0/8	**4/5**	**7/10**	0/7	0/8	0/5	0/10	+	0
Malaga Lake Pk/ 6/28 Salem County N 39^0 34.500' W 75^0 3.380'	0/5	**1/10**	**1/6**	**1/9**	0/5	**2/10**	**6/6**	**7/9**	0/5	**1/10**	**1/6**	**1/9**	0	0
Spruce Run Res./ 6/29 Hunterdon County N 40^0 39.685' W 75^0 55.964'	**1/8**	0/7	**1/7**	**1/8**	0/8	0/7	**1/7**	0/8	0/8	0/7	0/7	0/8	+	+
Swartswood Lk. / 6/29 Sussex County N 41^0 4.387' W 74^0 49.212'	0/7	**1/8**	**3/10**	0/5	**3/7**	**2/8**	0/10	**1/5**	0/7	0/8	0/10	0/5	0	0
Shepard's Lake/ 7/1 Passaic County N 41^0 8.252' W 74^0 13.851'	0/11	0/9	0/6	0/4	**2/11**	**1/9**	0/6	0/4	0/11	0/9	0/6	0/4	0	0

LOCATION/DATE	Prevalence of **Cryptosporidium** positive geese by age-sex class				Prevalence of **Giardia** positive geese by age-sex class				Prevalence of **Crypto** and **Giardia** positive geese by age-sex class				**WATER**	
Latitude/Longitude	A-M	A-F	J-M	J-F	A-M	A-F	J-M	J-F	A-M	A-F	J-M	J-F	Crypto.	Giardia
Van Saun Zoo/ 7/1 Bergen County N 40^0 55.588' W 74^0 2.866'	**1/8**	0/7	0/10	**2/5**	0/8	0/7	0/10	0/5	0/8	0/7	0/10	0/5	NS	NS
Forsythe NWR/ 7/2 Atlantic County N 39^0 28.131' W 74^0 25.154'	0/11	0/4	**1/12**	0/3	**2/11**	**2/4**	**5/12**	**2/3**	0/11	0/4	**1/12**	0/3	+	0
Tuckerton/ 7/2 Ocean County N 39^0 36.237' W 74^0 20.806'	0/9	**1/6**	0/11	0/4	0/9	0/6	**1/11**	**1/4**	0/9	0/6	0/11	0/4	0	0
Johnson Park/ 7/7 Middlesex County N 40^0 39.370' W 74^0 27.100'	0/4	**5/11**	**1/9**	0/6	**1/4**	**2/11**	**1/9**	**2/6**	0/4	**1/11**	0/9	0/6	river + pond +	river + pond 0
Mercer G.C./ 7/7 Mercer County N 40^0 16.417' W 74^0 39.361'	0/10	0/5	0/7	0/8	0/10	0/5	0/7	0/8	0/10	0/5	0/7	0/8	+	0
Bernham Park/ 7/8 Morris County N 40^0 47.842' W 74^0 29.789'	0/7	**1/8**	**2/8**	0/7	**1/7**	0/8	**2/8**	**1/7**	0/7	0/8	**1/8**	0/7	0	0
Loantaka Park/ 7/8 Morris County N 40^0 46.138' W 74^0 27.362'	**1/10**	**1/17**	0/0	**3/3**	0/10	**1/17**	0/0	**1/3**	0/10	0/17	0/0	**1/3**	0	0
Rahway River Pk/ 7/8 Union County	0/9	0/6	**4/8**	**3/7**	0/9	0/6	**1/8**	**1/7**	0/9	0/6	0/8	0/7	river 0 lake 0 *	river 0 lake 0

LOCATION/DATE	Prevalence of **Cryptosporidium** positive geese by age-sex class				Prevalence of **Giardia** positive geese by age-sex class				Prevalence of **Crypto** and **Giardia** positive geese by age-sex class				WATER	
Latitude/Longitude	A-M	A-F	J-M	J-F	A-M	A-F	J-M	J-F	A-M	A-F	J-M	J-F	Crypto.	Giardia
N 40^0 37.154' W 74^0 17.145'														
Takanassee Lake/ 7/9 Monmouth County N 40^0 16.670' W 73^0 59.718'	**2/13**	**1/7**	**3/10**	**1/10**	**2/14**	0/7	**2/9**	0/10	0/14	0/7	**1/9**	0/10	+	0
Bucks Mill Rec A/ 7/9 Monmouth County N 40^0 17.425' W 74^0 12.147'	0/11	**1/9**	**1/8**	**4/12**	0/11	**1/9**	**2/8**	**2/12**	0/11	0/9	0/8	**1/12**	+	0
TOTAL	**5/137**	**12/130**	**17/125**	**15/108**	**11/137**	**12/130**	**27/125**	**25/108**	**0/137**	**2/130**	**4/125**	**3/108**	**7 +**	**3 +**
AGE CLASS TOTAL	**17/267**		**32/233**		**23/267**		**52/233**		**2/267**		**7/233**			

* microsporidium detected in the lake