National Wildlife Health Center

Screening for potential human pathogens in fecal material deposited by resident Canada geese on areas of public utility

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COMPLETION REPORT

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Background and Justification:

Resident populations of Canada geese (*Branta canadensis*) and ducks have increased to high numbers, particularly in urban and suburban areas, throughout the United States and in other countries. The earliest complaints related to expanding goose populations, from the 1940's to the 1970's, were mainly damage to crops in agricultural areas (Kahl & Samson, 1984; Conover & Chasko, 1985; Flegler, et al., 1987). In the late 1970s and 1980s, populations of Canada geese using urban and suburban areas increased as did the number of complaints related to the nuisance created by excess fecal material. Goose feces have been implicated in eutrophication of small ponds and lakes as well as contamination of school yards, parks and boating and swimming areas (Conover & Chasko, 1985; Cooper & Keefe, 1997). An overriding concern of the public in most areas with high densities of Canada geese is the possibility of disease transmission to humans from direct contact with fecal material or contaminated water (Conover & Chasko, 1985; Allan, et al., 1995; Cooper & Keefe, 1997; Smith, et al.,1999; Feare, et al., 1999). Local, State and Federal Wildlife Agencies receive numerous requests for information on diseases of Canada geese and ducks that are transmissible to humans. These same agencies receive annual requests for reduction in numbers or complete removal of waterfowl based in part on concerns for public health.

Several studies have been conducted to detect the presence of bacterial pathogens in fecal material of migratory waterfowl. *Campylobacter jejuni*, which causes acute diarrhea in humans, was isolated from caeca of 154 (35%) of 445 ducks killed by hunters in Colorado (Luechtefeld, et al., 1980). During a banding study in Washington, Pacha et al. (1988) collected cloacal swabs from ducks and recovered *Campylobacter* spp. from 82 (73%) of 113 samples. In addition, they collected recently deposited fecal material from fields with flocks of Canada geese and sandhill cranes (*Grus canadensis*); *Campylobacter* spp. were isolated from five of 94 (5%) fecal samples from Canada geese and 74 of 91 (81%) fecal samples from sandhill cranes (Pacha, et al., 1988). An earlier study by Hill and Grimes (1984), in the Wisconsin-Minnesota region of the upper Mississippi River, found no *Campylobacter* spp. in 50 cecal samples from ducks killed by local hunters. *Campylobacter* spp. have been previously isolated from other birds (Waldhalm, et al., 1964; Smibert, 1969; Simmons & Gibbs, 1977; Knill, et al., 1978; Luechtefeld, et al., 1980; Fenlon, 1981; Skirrow, 1982; Kapperud & Rosef, 1983; Hill & Grimes, 1984),

Listeria spp. have been isolated from avian species including geese (Gray, 1958; Seeliger, 1961), however the geese typically mentioned in studies were domestic species. Isolation of *Listeria* spp. from wild Canada geese has not been documented although other species of wild birds have been reported as having *Listeria* spp. in their feces (Weis & Seeliger, 1975; Fenlon, 1985; Gautsch, et al., 2000). To investigate the possibility of *Listeria* spp. transfer from seagulls and rooks to silage, a study was conducted in Scotland to compare the presence of *Listeria* spp. in feces collected from gulls feeding at sewage treatment facilities with feces collected from gulls resting at other sites (Fenlon, 1985). Samples from 26 of 99 (26%) gulls using sewage sites were positive for *Listeria* spp. and 15 of 99 (15%) were positive for *L. monocytogenes*. At non-sewage sites, 14 (8%) gulls were positive for *Listeria* spp. with only 8 (5%) positive for *L. monocytogenes*. This study indicates that exposure to sewage was a possible source of these pathogens.

The natural reservoir for salmonellae is the intestinal tract of warm-blooded and cold-blooded animals. Most infected animals, however, seem to be subclinically ill excretors of salmonella. Most cases of human salmonellosis are the result of ingesting food, water, or milk contaminated with animal wastes and are manifested by gastroenteritis. Although human salmonellosis is usually self-limiting in healthy adults (though septicemia can occur), lost time from work and the usual involvement of many people in outbreaks can cause significant economic losses. *Salmonellae* have been shown to be able to survive in the environment for at least nine months (Quinn, et al.,1994) providing for increased dissemination potential. In the Czech Republic*, Salmonella* spp. were found in 1 of 8 gulls using sewage treatment ponds and 4% of 189 adult black-headed gulls (*Larus ribibundus*) and 19% of their young collected from other bodies of water (Cizek, et al., 1994). *Salmonella* Typhimurium was identified in 2% of 849 herring (*L. argentatus)*, black-headed , common (*L. canus canus*), black-backed (*L. marinus*) and lesser black-backed gulls (*L. fuscus*) using a Copenhagen dump (Nielsen, 1960). In a two-year study in New Jersey, Bigus (1996) isolated eight *Salmonella pullorum* isolates from Canada geese. In a study of waterfowl conducted in the Chesapeake Bay area of Hussong et al. (1979) reported only 44 samples were tested from migratory waterfowl and no *Salmonella* spp. were recovered; the total number of samples from geese was not specified. Johnson and du Moulin (unpublished report, L.C. Johnson and G.C. du Moulin, Beth Israel Hospital, Boston, MA) cultured 72 intestinal samples from 18 geese collected at three different sites in eastern Massachusetts during one summer and were unable to recover any *Salmonella* or *Shigella* spp. Although some authors have attempted to link the occurrence of *Salmonella* spp. in wild birds with the transmission of

Salmonella spp. in domestic animals (Williams, et al., 1977; Hatch, 1996), to our knowledge, conclusive evidence, that includes DNA studies, is not available.

Escherichia coli is a member of the fecal coliform group and is considered a normal inhabitant of the intestinal track of all mammals and others, including Canada geese (Hussong, et al., 1979). Concern over *Escherichia coli* contamination, particularly when reported as high fecal coliform counts in recreational waters, is typically related more to its presence in feces and index of potential presence of other more serious pathogens, such as *Salmonella* and *Vibrio cholera*, than concern over inherent *Escherichia coli* pathogenicity. In the last few years, however, several well-documented food borne outbreaks occurred that were traced to strains of *Escherichia coli* capable of producing severe diarrhea and kidney damage leading to death in some immunocompromised or young people. The most well documented toxigenic *Escherichia coli* is serotype O157:H7 which belongs to the shiga toxin producing group, one of the four groups of *Escherichia coli* that are capable of causing illness. There are currently at least 112 serotypes of shiga toxin producing *Escherichia coli* (Bopp, et al., 1999). Feare et al. (1999) collected 50 swabs of fecal material from Canada geese the summer of 1993 at six parks in London, England and the summer of 1994 at twelve sites throughout England. Samples collected in 1993 contained potentially pathogenic organisms, including *Escherichia coli* (Class 1), *Enterobacter cloacae*, *Salmonella* spp., *Aeromonas hydrophilia* and *Providencia alcalifaciens,* in 6% to 44% of the samples. In 1994, samples collected at each of the 12 sites had bacteria that were potentially pathogenic; no *Campylobacter* spp. were found in 1993 or 1994. Although reports of *Escherichia coli* of serotype O157:H7 from deer have been reported by Rice et. al. (1995), other reports from wildlife are rare (Wasteson, et al., 1999). In another study, Hussong et al. (1979) examined a random selection of *Escherichia coli* from waterfowl and seven isolates of enterotoxin-producing *Escherichia coli* were identified but further details were omitted.

The ability of geese to act as transport or mechanical vectors for parasites was tested by Graczyk et al. (1997) by dosing Canada geese orally with *Cryptosporidium parvum* oocysts and subsequently monitoring feces for the presence of oocysts. In a follow-up study, Graczyk et al. (1998) collected fecal material of Canada geese during the winter at nine sites in Maryland; *Cryptosporidium* spp. oocysts were present in samples from seven of nine sites and *Giardia* spp. cysts were present in samples from all nine sites. *Cryptosporidium parvum* was identified in Canada goose feces from one site by using a mouse bioassay and by polymerase chain reaction (PCR), a molecular detection method. The mouse bioassay allowed Graczyk et al. (1998) to test if oocysts that passed through the gut would remain viable and infectious. Although *Cryptosporidium parvum* is not pathogenic to birds, presence of this organism suggests that it could be transmitted to mammals through contamination of drinking water. It should be noted that *Cryptosporidium baileyi* and *Cryptosporidium meleagridis* as well as species of *Giardia* and which can infect and multiply within geese, however, these species are not human pathogens (Darabus, 1997; Morgan, et al., 2000).

Skene et al. (1981) conducted a study at a waterfowl park and sanctuary in Ontario to detect the presence of coccidia in freshly deposited fecal material collected from randomly selected adult Canada geese during winter months and fecal material collected from newly hatched goslings from five families in the spring. They confirmed low numbers of coccidia in 21 (20%) of 104 samples from adult geese. Goslings from 3 of 5 families were shedding oocysts within eight days of hatching. Adult geese shed *Eimeria magnalabia* (3%), *Eimeria hermani* (14%), *Eimeria truncata* (2%), and *Tyzzeria parvula* (2%). Goslings only shed *Eimeria hermani* but the presence of oocysts within eight days of hatching indicated availability of oocysts on soil. *Eimeria* spp. and *Isospora* spp. are very host specific; *Isospora belli* is the only known human

pathogen (Koneman, et al.,1997).

Chlamydia, rotavirus, and avian influenza virus are all well described human pathogens. Avian influenza infection occurs in a variety of wild and domestic bird species with the outcome ranging from no obvious clinical signs to 100% mortality (Swayne, et al., 1998). A 1997 occurrence of avian influenza in Hong Kong involved 18 human cases (Snacken, et al., 1999) and raised concerns about transmission of avian influenza from birds to humans (Webster, et al., 1993). Rotaviruses are capable of causing gastroenteritis in the young of mammalian (Endtz, et al., 1991) and avian species (Stott, 1999). *Chlamydia psittaci* is capable of causing serious or fatal disease in most birds and mammals including humans (Grimes, et al., 1979; Wobeser & Brand, 1982; Brand, 1989; Franson & Pearson, 1995; Grimes, et al., 1997). *Chlamydia psittaci* has been isolated from at least 159 bird species including waterfowl (Friend and Franson,1999).

In addition to viruses that pose a risk to human health, isolation and identification of Newcastle disease virus and duck plague virus was included because they are diseases of importance to wild birds and domestic poultry and waterfowl (Awan, et al., 1994). Newcastle disease virus is one of the most important pathogens for birds of all types but the only known outbreaks have occurred in double crested cormorants (Kuiken, et al., 1998; Kuiken, et al., 1998; Glaser, et al., 1999). Duck plague only occurs in ducks, geese and swans. It has been isolated from many areas in the United States (Converse & Kidd, 2001).

Objective:

The objective of this study was to determine if specific organisms that may cause human disease are present in fecal material deposited by resident populations of Canada geese in urban and suburban environments. Specifically we:

1. Determined the presence of bacteria, viruses, and parasites in fresh and accumulated fecal material deposited by Canada geese.

2. Evaluated the findings relative to risk of exposure to diseases for humans and animals is association with fecal material deposited by Canada geese.

3. Compared the presence of any pathogen in fecal samples collected 24 hours after raking the transect, with samples marked and collected five days later on the same transect.

Methods and Procedures:

Study areas and fecal collection methods

Twelve study sites were selected in the northeastern and mid-Atlantic states of Massachusetts, New Jersey and Virginia, within Region 5 of the U.S. Fish and Wildlife Service (USFWS). In each state, four areas were selected that had daily use by resident Canada geese as well as frequent use by the public (**Figure 1**). In Massachusetts, the Massachusetts Division of Wildlife selected a Audubon Sanctuary with a grassy area next to an office building and parking lot and a second grassy area across from a picnic area in a former U.S. Army base. Also in Massachusetts, two town parks were selected by the U.S. Department of Agriculture Wildlife Services. In New Jersey, sites selected by the New Jersey Division of Fish and Wildlife included a municipal park in a residential area with a children's playground and picnic area; a park with two lakes, picnic areas, recreational sports field, a

petting zoo, and a horse track; a park along the Delaware River with picnic areas, and playgrounds; and a municipal park with a lake, picnic areas and hiking trails. In Virginia, the Virginia Department of Game and Inland Fisheries selected areas that included a group of summer condominiums with several small lakes surrounded by mown grass; an area along a lake adjacent to a small shopping area and restaurant; a park with hiking trails, food concession, swimming and boat rentals; and a summer camping site for trailers with a swimming pool and a lake.

At each study site, two to four transects were established that measured 2 to 4 m wide by approximately 20 meters long to encompass a total area of 160 m^2. The exact configuration of transects varied in correspondence with the shape of the areas used by geese. Once the transects were established, they were raked to remove accumulations of fecal and other debris material prior to sample collection. Within 24 hours after site preparation, 10 samples of freshly deposited goose fecal material were collected using rubber gloves, placed in pre-labeled plastic bags, and held on ice in coolers during transport. The samples from the four sites were packed in coolers with blue ice for coolant, and shipped to NWHC via overnight courier service. At least five additional samples of fresh feces on the transect area were marked using # 17 nails sprayed with bright orange paint. After five days, each site was revisited and marked samples were collected and submitted following the same methods. Samples were collected along transects three times, no less than 20 days apart, to provide a temporal aspect to the study. On receipt at NWHC, fecal samples were subdivided and distributed to the bacteriology, parasitology, and virology laboratories. The remaining fecal material was frozen at a minimum of -40C for subsequent study if necessary.

Bacteriological survey

Bacterial analysis was attempted to identify the following organisms: *Salmonella* spp., *Campylobacter* spp., *Listeria* spp*.,* and *Escherichia coli* O157:H7. Approximately one gram of each fecal sample was placed into an appropriate enrichment broth for each organism of interest in this study as follows:

- For isolation of *Salmonella* spp., Rappaport-Vassiliadis (Dusch & Altwegg, 1995) and dulcitol-selenite broths (Raj, 1966) were incubated at 43C for 18-24 hours before being inoculated onto XLT4 and brilliant green agars and incubated at 37C for 18-24 hours (Bopp, et al., 1999).
- For isolation of *Escherichia coli* O157:H7, *Escherichia coli* enrichment broth was incubated at 37C for 18-24 hours before being inoculated on MacConkey with sorbitol agar (March & Ratnam, 1986; Bopp, et al., 1999).
- For isolation of *Listeria* spp., UVM and PALCAM listeria enrichment broths were incubated at 30C for 18-24 hours before being inoculated on LPM, PALCAM, and Oxford agars (Gunasinghe, et al., 1994).
- For isolation of *Campylobacter* spp., *Campylobacter* thioglycolate enrichment broth was incubated at 42C under microphilic conditions for 18-24 hours, inoculated on CCDA and CampyCVA agars and incubated at 37C for 18-24 hours (Endtz, et al., 1991; Nachamkin, 1999).

Media used for enrichments and isolations were purchased commercially (Remel, Lenexa, KS or Becton Dickinson, Cockeysville, MD) or prepared at NWHC from commercial materials.

All bacterial colonies present after the incubation period were screened for organisms based

on typical colony morphology and biochemical reactions on selective or differential media. Isolates matching preliminary biochemical and morphological properties of organisms of interest were further biochemically characterized and identified using an appropriate API system (bioMerieux, St. Louis, Missouri). Isolates that conformed to biochemical characteristics consistent with bacterial pathogens of interest were subcultured to tryptic soy broth with glycerine and frozen at -70C as reference stock. Further characterization by a secondary identification system (Biolog, Hayward, California) was attempted on bacterial colonies that were refractory to initial identification by the primary identification system and suspected of being bacterial pathogens of interest. All identification systems were used according to manufacturer's recommendations and standard protocols.

Isolates tentatively identified as *Salmonella* species were serotyped by the National Veterinary Services Laboratory (Ames, IA). Isolates of *Escherichia coli* that were sorbitol negative were tested using the RIM *Escherichia coli* O157:H7 latex agglutination kit (Remel, Lenexa, KS).

All organisms identified as *Listeria* spp. were further characterized (**Table 1**). For the beta lysin test, disks impregnated with *Staphylococcus aureus* beta lysin (Beta Lysin Disk, Remel, Lenexa, KS) were used as a surrogate to *Staphylococci* CAMP test (Bille, et al., 1999). Sugar fermentation tubes were purchased commercially (Remel, Lenexa, KS). A molecular fingerprinting method was used to further characterize 1/4 of the *Listeria* spp. isolates by ribotyping using a commercial application of the technology called the RiboPrinter™ (Qualicon, Wilmington, DE). The evaluation by RiboPrinter was done under contract with Cornell University, Laboratory of Molecular Typing (Ithaca, NY). All *Salmonella* isolates were also fingerprinted using the RiboPrinter.

Several samples were rechecked following initial examination. An additional assay was used to recheck 40 samples for any evidence of *Campylobacter* spp. using ProSpecT *Campylobacter* Microplate Assay (Alexon-Trend, Ramsey, MN). The ProSpecT *Campylobacter* Microplate Assay is an enzyme-linked immunosorbent assay (ELISA) that detects specific antigen from *Campylobacter* spp. but is not species specific. Samples were also rechecked for evidence of *Campylobacter, Shigella, Yersinia, Salmonella,* and *Escherichia coli* O157:H7. using a PCR based kit (Multi-enteric 5; Vita-Tech Laboratories, Ontario, Canada). The procedure included DNA extraction, specific DNA amplification, and visual detection of amplification products.

Parasitological surveys

The parasites of existing concern to human health initially selected for this study included *Cryptosporidium*, *Giardia,* and *Cyclospora* spp.. A subsequent decision to not attempt isolation of *Cyclospora* spp. was made prior to initiation of the sampling. The air handling system needed during the 6-14 day period necessary for sporulation of this organism was not available and the health risk for NWHC personnel could not be mitigated.

Each sample was diluted with 30 ml of tap water, mixed on a vortex mixer, filtered through two layers of cheese cloth and centrifuged at 20,000 rpm for 15 min. Supernatent was removed and the pellet was resuspended in 10 ml of 10% neutral buffered formalin. Examination of samples for *Cryptosporidium* and *Giardia* spp. were done using Meriflour (Meridian Diagnostics, Inc., Cincinnati, OH) per kit instructions. For a sample to be considered positive, it was examined for fluorescence, internal and external morphology (the latter two conducted with Nomarski-type interference contrast microscopy), and size

measurements. If no organisms were seen, the sample was designated as a no parasites seen. The methods employed allowed spiked samples with 325 oocysts of *Cryptosporidium parvum* per milliliter to be detected. Below 325 oocysts/ml our test did not yield satisfactory results. Compared to RT-PCR used by Graczyk et al. 1998, the immunofluorescence assay is not as sensitive but achieved satisfactory results.

Virological surveys

Isolation and detection of viruses were attempted for potential human pathogens that included avian influenza virus, rotaviruses, and *Chlamydia psittaci*. Although *Chlamydia psittaci* is a bacteria-like organism, virology cell culture technique is most often used to isolate the organism which is included under the virology survey portion of this study.

Isolation of avian influenza virus was attempted using embryonated hen's eggs (Senne, 1998). Identification and pathogenicity determination were accomplished using methods described by Swayne, et al. (1998). A PCR test (Vita-Tech Laboratories, Ontario, Canada), designed to detect *Chlamydia psittaci,* was used according to the manufacturer's instructions. Rotaviruses were detected using an Eminase test, according to the instructions in the kit (Pro-Lab Diagnostics, Austin, Texas).

Newcastle disease viruses were cultured using methods described by Alexander (1998). The nested PCR method of Hansen, et al. (1999) was used to detect duck plague virus. Isolation of duck plague virus was also attempted in Muscovy duck embryo fibroblast cell culture prepared as described by Docherty and Slota (1988).

Results and Discussion:

Samples were collected in New Jersey between July 15 and September 13, 1999 and in Virginia between July 22 and September 17, 1999 (**Figure 2**). Massachusetts was selected to replace the third state originally selected for the study. The sample collection in Massachusetts was delayed slightly due to this change; samples were collected between August 25 and November 1, 1999 at two sites and September 7 and November 4, 1999 at the other two sites (**Figure 2**). A severe drought continued throughout this time period at all New Jersey and Virginia study sites. Most of the 5 day samples were completely desiccated and had to be pulverized with a mortar and pestle prior to hydration and examination. The desiccated 5 day samples were not suitable for virus isolation. As a result of the drought, the protocol for the second and third collections was modified slightly. Cooperators were instructed to place a small amount of the 24 hour fecal sample in each of two transportation media, Cairy Blair and Buffered Glycerol. This method was selected in an attempt to improve isolation of some organisms particularly *Campylobacter* spp. but was not noticeably effective. At the Johnson Park site in New Jersey, the first set of samples was collected next to a small pond used by the Canada geese as a late afternoon and evening roost site. This pond continued to recede and was dry by the second sampling period. The geese moved to another pond in the park. Therefore, a new transect line was established at this pond for collection of the second and third set of samples. Hurricane Floyd caused heavy rainfall in New Jersey between the 24 hour and 5 day sample collections early in September and washed away the 5 day samples in the third collection period. In Massachusetts, there was normal rainfall in October and the 5 day samples retained enough moisture to allow for culture for bacteriology and virology. Unfortunately by late October, Canada geese were no longer using the Devens area in Massachusetts and no samples were available for the third collection.

Bacteriology

Salmonella spp.:

Salmonella was isolated from two samples; *S.* Typhimurium from an October (24 hour) sample collected at the Brockton Massachusetts site and *S.* Hartford from an August (5 day) sample collected at the Van Suan New Jersey site (**Table 2**). *Salmonella* Typhimurium typically accounts for 20% of human cases (Koneman, et al.,1997) and about 28% of wild bird isolates (NWHC unpublished data). In contrast*, S.* Hartford is a rarer isolate with little information on its role in human disease. It was implicated in a 1996 food poisoning event in New York that was linked to an improperly dug and protected well for drinking water (MMWR 1998. 47(19):394-396). While isolation of *Salmonella* spp. from Canada geese is a rare event (<0.5%), potential transmission to humans should be considered. Further studies to refine incidence in other populations of Canada goose should be considered.

Escherichia coli, serotype O157:H7

No *Escherichia coli* of serotype O157:H7 was isolated. There were six sorbitol negative *Escherichia coli* isolations from three samples but serological screening ruled out serotype O157:H7. This is the first study to sample Canada geese specifically for evidence of *Escherichia coli* O157:H7. While this sample is small compared to the number of urban Canada geese, the potential for transient carriers of *Escherichia coli* O157:H7 needs to be further studied to completely rule-out involvement of Canada geese in transmission of this pathogen.

Campylobacter spp.

No *Campylobacter* spp. were isolated. The subset of samples tested using the ELISA method were also negative for evidence of *Campylobacter* spp. (**Figure 2**). Only two reports on *Campylobacter* spp. in geese have been published. Pacha et al. (1988) found a low incidence of samples from geese positive for *Campylobacter* spp. (5%); and Taylor et al. (1983) had two Canada goose samples positive for *Campylobacter* spp. but they did not state the overall frequency of positive samples in their study.

Listeria spp.

Listeria spp. were isolated from 47 of 495 samples (7%); 38 were 24 hour samples and nine were 5 day samples. The majority of the isolates were either *Listeria innocua* (~55%) or *L. monocytogenes* (~42%)(**Table 3**). There was one isolate each of *L. grayi,* and *L. welshmeri* which are typically not regarded as pathogenic (Quinn, et al.,1994; Low & Donachie, 1997) although their specific pathogenicity in geese has not been studied. This isolation rate is consistent with Wesley's (1999) study that showed a 9.5% prevalence rate for *L. monocytogenes* in healthy ring-billed gulls. This study does not suggest a 7% prevalence rate in geese, however, since it is possible that some fecal samples may have come from the same goose. Isolation of *Listeria* spp. from the 5 day-old samples is also consistent with the study by Fenlon (1999) that reports *L. monocytogenes* is able survive in the outside environment for more than two years in domestic animal feces. While most of the isolates obtained from wild birds in earlier works have been identified as *Listeria monocytogenes*, there were only two species within *Listeria* until the mid-1960s (Rocourt, 1999). This limitation causes us to question the exact species isolated by Fenlon (1999) as compared to this report's findings. In this study, *L. monocytogenes* constituted less than half of the isolates

where as *L. innocua* (recognized as a species in 1981) was the slightly predominate isolate. *L. innocua* is considered to be nonpathogenic to humans and animals (Low & Donachie, 1997; Rocourt, 1999). Isolation of *Listeria* spp. from Canada geese is consistent with literature that implicates *Listeria* spp. as an environmental organism that is commonly associated with grasses and silage (Low & Donachie, 1997). It seems consistent with Low's study to isolate *Listeria* spp. from otherwise healthy Canada geese given they are herbivores.

Although serotyping was not done on the *Listeria* spp., results of the RiboPrinter (**Figure 3**), indicate one *L. monocytogenes* has the same genetic fingerprint as *L. monocytogenes* serotype 1/2b (Nadon, et al., 2000). *L. monocytogenes* serotypes 1/2a, 1/2b, and especially 4b have been implicated as causative strains involved in human diseases (Clarridge, 1987; Bille, et al., 1999). Other genetic fingerprints of *L. monocytogenes* could include other pathogenic serotypes, including 4b, however discrimination is not sufficient without further study.

Parasitology

Cryptosporidium and *Giardia* spp.

Fecal samples from two sites in Massachusetts were positive for *Cryptosporidium* and *Giardia* spp. and fecal samples from one site in New Jersey and one site in Virginia were positive for *Giardia* spp. (**Table 5**). Based on the size of the *Cryptosporidium* spp*.* we suspect it was *Cryptosporidium parvum*. Samples from several sites had organisms present which fluoresced and were within the size category for identification as *Giardia* spp. Upon examination of the internal structures of these organisms, however, neither a nuclei nor an axostyle were seen. A sporoblast was present which would be indicative of an *Eimerian* or *Isosporan* coccidia. As noted earlier, *Eimeria* spp. and *Isospora* spp. found in geese and ducks are not infectious for humans. To help elucidate this problem, goose fecal samples are being collected for sporulation that will allow internal structures to develop. These samples will be examined by the Meridian kit for cross reactivity. This observation of flourescent organisms, that are not known pathogens to humans, highlights the importance of careful examination and measurements of shape, size and internal structures in making a positive identification, not just fluorescence alone.

Virology

Chlamydia psittaci

Chlamydia psittaci was detected in 11 individual bird samples (24 hour) from Massachusetts and New Jersey and two, pooled (5 day) samples of feces from Massachusetts (**Table 4**). The detection of *Chlamydia psittaci* in 13 fecal samples is a significant finding as the presence of this pathogen could indicate a risk to human health. The PCR test used detects both infectious and noninfectious *Chlamydia psittaci*. Our experience culturing the *Chlamydia psittaci* organism, since the initiation of this study, is that the chances of isolation are greatly increased when the sample is placed directly in a transport medium in the field. Although the samples for this study were collected in a satisfactory way for the PCR detection of *C. psittaci*, experience has taught us that a number of false negatives will be generated if isolation is attempted using those same samples. The best follow-up would be the collection of new samples in selected areas with the collection method specifically addressing the isolation of *C. psittaci*. Now that we have the necessary PCR technology available, follow-up research studies should be done in a new study to determine whether the infectious form is

present at these sites.

Rotavirus

A rotavirus was detected in one (24 hour) fecal sample collected at the Rockahock site in Virginia (**Table 2**). The method used in this study detected rotaviruses at the level of genus, which means that rotaviruses pathogenic for both mammalian and avian species were potentially detected. Rotaviruses are generally difficult to isolate in cell culture, and the one positive sample was no exception. The virus could not be isolated so additional follow-up to determine the species was not possible.

Paramyxovirus

Newcastle Disease Virus and avian influenza virus were not detected in fecal samples tested. Hemagglutinating agents (Alexander, 1998), other than NDV, were isolated from nine (24 hour) fecal samples from the Norfolk and Arlington sites in Maryland and one pooled fecal sample from a New Jersey site (**Table 6**). Because nonpathogenic paramyxoviruses are frequently isolated from avian intestinal tracts these isolates are considered inconsequential to this study.

Duck plague virus

Duck plague virus was not present in any of 80 fecal samples tested. Based on results from a 1998 study of duck plague conducted in Maryland where ~11% positivity was found in free flying waterfowl (USGS NWHC unpublished data), the sample number from each site in this study may have been too small to detect geese shedding duck plague virus.

Conclusions:

This study was done to determine the presence of some selected organisms that could cause disease in humans exposed to fecal material of Canada geese collected at sites with a history of high public use and daily use by Canada geese in the northeastern United States. The methods used for transect delineation, site preparation, and sample collection, preservation and transportation were very successful. Attempts to isolate four bacterial organisms resulted in no isolates of *Campylobacter* spp. or *Escherichia coli* O157:H7; two isolates of *Salmonella*, one *S.* Typhimurium and one *S.* Hartford; and forty-seven isolates of *Listeria* spp., including 13 isolates of *Listeria monocytogenes*. Attempts to detect two viruses and chlamydia resulted in no isolation of paramyxovirus; one detection of a rotavirus, and 13 samples that are suspected to contain *Chlamydia* spp. Parasitological examinations resulted in detection of four samples with *Giardia* spp. and three samples with *Cryptosporidium* spp. (**Table 6**).

Bacteria and viruses were successfully isolated in 24 hour and 5 day samples. There were decreasing numbers of samples positive for bacteria in five day samples, particularly in the second and third sample periods as drought conditions continued. A rotavirus was detected in a 24 hour sample and a total of 13 *Chlamydia psittaci* positive samples were detected in both 24 hour and 5 day samples. Eleven *Chlamydia psittaci* positive samples were detected in those collected after 24 hours while only two were detected after 5 hours. The detection methods used in this study do not differentiate between infectious and noninfectious *Chlamydia psittaci* or rotaviruses. Both of these agents, in an infectious state, pose a serious human health threat. As soon as possible further field and laboratory studies should be

carried out to determine whether the fecal material, found where urban Canada geese congregate, contains infectious *Chlamydia psittaci* or rotaviruses..

These was no consistent distribution of positive samples over time, within sample periods or geographic locations (**Table 7**). Low frequency of positive cultures indicate that risk of humans to disease through contact with Canada goose feces appeared to be minimal at the four sites in Massachusetts, New Jersey and Virginia during the summer and early fall of 1999. We suggest further studies be conducted in other areas with resident Canada geese during different seasons to detect differences in prevalence and survival of organisms.

Acknowledgments

Disclaimer

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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