CHARACTERIZATION OF CAMPYLOBACTER FROM RESIDENT CANADA GEESE IN AN URBAN ENVIRONMENT

M. Elizabeth Rutledge,1,4 Robin M. Siletzky,2 Weimin Gu,2 Laurel A. Degernes,3 Christopher E. Moorman,1 Christopher S. DePerno,1 and Sophia Kathariou2
1 Fisheries, Wildlife, and Conservation Biology Program, Department of Forestry and Environmental Resources, North Carolina State University, Campus Box 7646, Raleigh, North Carolina 27695, USA
2 Department of Food, Bioprocessing, and Nutrition Sciences, North Carolina State University, Campus Box 7624, Raleigh, North Carolina 27695, USA
3 Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Campus Box 8401, Raleigh, North Carolina 27607, USA
4 Corresponding author (email: merutled@ncsu.edu)

ABSTRACT: Waterfowl are natural reservoirs for zoonotic pathogens, and abundant resident (nonmigratory) Canada Geese (Branta canadensis) in urban and suburban environments pose the potential for transmission of Campylobacter through human contact with fecal deposits and contaminated water. In June 2008 and July 2009, we collected 318 fecal samples from resident Canada Geese at 21 locations in and around Greensboro, North Carolina, to test for Campylobacter. All campylobacter species detected were C. jejuni isolates, and prevalences in 2008 and 2009 were 5.0% and 16.0%, respectively. Prevalence of C. jejuni-positive sampling sites was 21% (3/14) and 40% (6/15) in 2008 and 2009, respectively. All C. jejuni isolates were susceptible to a panel of six antimicrobial agents (tetracycline, streptomycin, erythromycin, kanamycin, nalidixic acid, and ciprofloxacin). We used pulsed-field gel electrophoresis and fla-typing to identify several strain types among these isolates. Multilocus sequence typing of representative isolates revealed six sequence types, of which two (ST-3708 and ST-4368) were new, two (ST-702 and ST-4080) had been detected previously among C. jejuni from geese, and two (ST-991 and ST-4071) were first reported in C. jejuni from an environmental water source and a human illness, respectively. These results indicate a diverse population of antibiotic-susceptible C. jejuni in resident Canada Geese in and around Greensboro, North Carolina, and suggest a need for additional assessment of the public health risk associated with resident Canada Geese in urban and suburban areas.

Key words: Branta canadensis, Campylobacter jejuni, resident Canada Geese, strain types, waterfowl, zoonotic disease.

INTRODUCTION

Resident (nonmigratory) Canada Geese (Branta canadensis) populations have grown in urban areas in North America, primarily because of increased availability of resources (e.g., open areas of grass and water) and lack of natural predators (McCoy, 2000). In 2008, the number of resident Canada Geese in the United States was estimated to be four million, four times the estimate in 1990 (Dolbeer et al., 2009). Increases in resident goose populations and the presence of fecal material may enhance the potential for transmission of infectious agents between geese and humans. Recreational areas (e.g., parks, corporate landscapes, golf courses) provide excellent habitats for geese, resulting in increased human-geese interactions. Campylobacter and other zoonotic pathogens can reside in the intestinal tract of birds (Aydin et al., 2001; Abulreesh et al., 2006; Van Dyke et al., 2010), and large amounts of feces (typically >0.45 kg of feces per day) produced by Canada Geese at recreational sites may constitute human health hazards (Kassa et al., 2001).

Campylobacter is one of the leading bacterial causes of human gastroenteritis, with 0.8 million cases annually in the United States (Scallan et al., 2011). Human gastroenteritis can be accompanied by severe autoimmune sequelae, including Guillain-Barré syndrome and Reiter’s syndrome (Nachamkin et al., 1998; Skirrow and Blaser, 2000; Gillespie et al., 2002). Approximately 85% of human cases are due to Campylobacter jejuni,
with the majority of the remainder involving *Campylobacter coli* (Friedman et al., 2000; Gillespie et al., 2002). *Campylobacter* can colonize (typically without symptoms) a large range of animal hosts, and contamination of poultry by this pathogen is considered a leading risk factor for human illness (Rosenquist et al., 2003).

The role of wildlife in human *Campylobacter* infections remains poorly characterized (Petersen et al., 2001; Hepworth et al., 2011; Jokinen et al., 2011). *Campylobacter jejuni* has been recovered from healthy waterfowl (Pacha et al., 1988; Fallacara et al., 2001, 2004; Abulreesh et al., 2006; Van Dyke et al., 2010), but genotyping has failed to yield clear links to human campylobacteriosis (Fallacara et al., 2001, 2004; Wahlström et al., 2003; Abulreesh et al., 2006). However, in 1994 and 1995, large outbreaks of campylobacteriosis (approximately 1,000 people) in Norway were attributed to drinking water sources contaminated with *C. jejuni* from the feces of Pink-footed Geese (*Anser brachyrhynchus*; Varslot et al., 1996). In 2008, an outbreak of human campylobacteriosis in Alaska was attributed to peas contaminated with *C. jejuni* from feces of Sandhill Cranes (*Grus canadensis*; Gardner et al., 2011).

Canada Geese have been recognized as contributors to water contamination and potential reservoirs for several pathogens, including *Campylobacter* (Pacha et al., 1988; Feare et al., 1999; Converse et al., 2001; Fallacara et al., 2001; Kassa et al., 2001; Van Dyke et al., 2010; Jokinen et al., 2011). However, the phenomenon of large resident populations of Canada Geese in urban and suburban settings in the United States is relatively recent, and limited data are available on prevalence and strain types of *Campylobacter* from these populations. In the summer of 2008 and 2009, we determined prevalence of *Campylobacter* from a resident Canada Goose population at several urban and suburban sites in and around Greensboro, North Carolina, USA. Additionally, we determined antimicrobial susceptibility of campylobacters and determined strain types via a combination of three strain-typing tools.

**MATERIALS AND METHODS**

We collected fresh fecal samples from resident Canada Geese at 14 sites between 16 and 18 June 2008 (n=218) and 15 sites on 27 July 2009 (n=100) in and around Greensboro, North Carolina (36°4'48"N, 79°56'59"W; Fig. 1). The geese were considered resident because only resident individuals are present in the region from March to August (USFWS, 2011). Sample collection sites included local lakes, parks, fields, corporate landscapes, golf courses, and residential areas (Table 1 and Fig. 1). The sites sampled and variability in the number of samples per site (5–45) reflect the number of geese present at each site during the sampling visit. We collected freshly voided fecal samples (based on wet appearance of the sample and direct observation of fecal droppings deposited by the birds) using a sterile tongue depressor, placed the samples in individual plastic bags, and stored them on ice in the field. Within 24 hr of collection, we took the fecal samples to the laboratory and stored them at 4°C until processing.

**Isolation of Campylobacter**

To isolate *Campylobacter* species, we plated fecal material (0.1 g) from each sample directly onto blood-free modified charcoal cefoperazone desoxycholate agar (CCDA; Oxoid, Hampshire, UK). We incubated the cultures at 42°C for 48 hr in a microaerobic environment generated by a GasPak EZ Campy sachet (Becton, Dickinson and Co., Sparks, Maryland, USA). We subcultured putative *Campylobacter* colonies on tryptic soy agar with 5% sheep blood (SBA; Remel, Lenexa, Kansas, USA) until a pure culture was obtained. We characterized one colony from each of the *Campylobacter*-positive samples from 2008 and, when possible, selected two colonies from each of the 2009 positive samples.

**Determination of Campylobacter species and antimicrobial susceptibility profiles**

We determined the species of each purified *Campylobacter* isolate by polymerase chain reaction (PCR; Smith et al., 2004) and tested the isolates for resistance against a panel of antibiotics including tetracycline, streptomycin, erythromycin, kanamycin, nalidixic acid,
and ciprofloxacin using the agar dilution method in serial 2-fold dilutions (Gu et al., 2009). Breakpoint values (μg/mL), previously described by Gu et al. (2009), included ciprofloxacin, ≥4; erythromycin, ≥8; kanamycin, ≥64; nalidixic acid, ≥32; streptomycin, ≥64; tetracycline, ≥16.

**Campylobacter strain typing**

To identify *Campylobacter* strain types among the isolates, we used fla-typing, pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing (MLST). For fla-typing, we amplified the flaA gene with the PCR using primers flaAF (ATGGGATTTCGTATTACAC) and flaAR (CTGTAGTAATCTTAAAA-CATTATTG; Smith et al., 2004). Digestion of the PCR product with DdeI, separation of the fragments, and analysis of the resulting image were conducted using BioNumerics (version 4.6; Applied Maths, Ibis Biosciences, Carlsbad, California, USA; Smith et al., 2004). We performed cluster analysis using the band-based Dice coefficient with a lane optimization of 2.0% and band tolerance of 2.0% to identify genetic relatedness among strain types.

We performed PFGE using Smal (Gu et al., 2009), and for cluster analysis we used the band-based Dice coefficient with a lane optimization of 1.5% and band tolerance of 1.5%. We generated a dendrogram (Fig. 2) using the unweighted-pair group method with arithmetic averages (UPGMA). To determine the allele profile and sequence type (ST), we performed MLST as described previously (Miller et al., 2006; Gu et al., 2009). The amplified products were sequenced by Genewiz, Inc. (Germantown, Maryland, USA). The sequences were analyzed using BioEdit (version 7.0, BioEdit Sequence Alignment Editor) and analyzed against the *C. jejuni/C. coli* MLST database (http://pubmlst.org/Campylobacter) (Jolley and Maiden, 2010).

**RESULTS**

We isolated *Campylobacter* at three of the 14 (21%) sites in 2008 and six of the 15
In 2009, samples from this same site yielded typical Campylobacter cultures (Table 1). Of the eight locations sampled in both years, two yielded Campylobacter each year. Of the remaining six sites sampled both in 2008 and 2009, four were negative in both years, and two were positive for Campylobacter only in 2009 (Table 1).

We isolated Campylobacter from 10 of 218 (5%) fecal samples in 2008 and 16 of 100 (16%) samples in 2009 (Table 1). Prevalence of Campylobacter-positive samples varied among the sites, from 7% (1/15) to 80% (4/5). A residential neighborhood site, Residential 1, had the highest prevalence of Campylobacter each year (34% and 80%, respectively). In 2008, isolates from two sites accounted for 90% (9/10) of those obtained. These same two sites contributed 39% of the isolates obtained the following year. We obtained positive samples from four additional sites in 2009, three of which had not been surveyed in 2008 (Table 1).

All Campylobacter isolates were identified as C. jejuni. Although some variation in the antibiotic minimum inhibitory concentrations (MIC) was noted among the isolates, the MIC was below the resistance breakpoint value for each compound (Gu et al., 2009). Genomic fingerprinting of the C. jejuni isolates by fla-typing and PFGE indicated that in the majority (78%) of the Campylobacter-positive sites, the Canada Geese were
colonized by one strain of *C. jejuni* at a given time. Only two sites yielded two strain types each, both in 2009. Although the Canada Goose populations from these two sites had a mixture of *C. jejuni* strain types, the two isolates from the same sample always had the same *fla* and PFGE profiles (Fig. 2).

Strain types detected among isolates in 2008 were distinct from those of *C. jejuni* isolates from 2009. In 2008, we identified three strain fingerprints: one in *C. jejuni* from Corporate 2 and Residential 1 (one isolate each), a closely related type (identical *fla* types and a single-band difference with PFGE) in four isolates from Residential 1, and a third strain type in all four Corporate 10 isolates. Although all strain types from 2008 could be distinguished by *fla*-PFGE from 2009 samples, there were pronounced similarities between the Residential 1 isolates from 2008 and half of

---

**Figure 2.** Dendrogram of Canada Geese *Campylobacter jejuni* isolates based on the combined profiles generated by pulsed-field gel electrophoresis (PFGE) using Smal and *fla*-typing from samples collected in and around Greensboro, North Carolina, 2008–09. Different clusters are indicated with different letters (A–D). Clusters A1, B1, and C1 were closely related to A, B, and C, respectively.
the isolates from the same site in 2009. These Residential 1 isolates from 2008 and 2009 shared the same fla type and had closely related PFGE profiles, differing only in the size of the largest band. Clearly distinct fla and PFGE profiles were exhibited by the remaining four isolates from Residential 1 in 2008 (Fig. 2).

Seven isolates (11957, 12022, 12156-1, 12184-2, 12188-1, 12189-1, and 12215-2) representative of distinct fla-PFGE profiles were analyzed by MLST. With the exception of 12215-2 and 12188-1, which were both of ST-4080 and had highly similar (92% identity) fla and PFGE profiles (Fig. 2), each of the other five isolates had different STs, which also belonged to different clonal complexes. The STs 3707 and 4368 were newly identified in this study.

DISCUSSION

In this study, resident Canada Geese shed C. jejuni. Although previous surveys provided evidence for the potential of Canada Geese to serve as reservoirs for Campylobacter (Pacha et al., 1988; Aydin et al., 2001; Wahlström et al., 2003; Van Dyke et al., 2010), limited data are available on Campylobacter strain types, genetic diversity, and antimicrobial susceptibility of C. jejuni from urban and suburban sites. We focused on C. jejuni and C. coli, as these are the Campylobacter species primarily responsible for human disease (Friedman et al., 2000; Gillespie et al., 2002).

We detected a prevalence of C. jejuni similar to that reported for migratory Canada Geese (5%; Pacha et al., 1988) but significantly lower than the 50% prevalence from birds in metropolitan parks in central Ohio (Fallacara et al., 2001). We found relatively low frequency of C. jejuni–positive samples from several of the C. jejuni–positive sites, despite close proximity of the individual geese and the observed abundance of fecal droppings. It is possible that colonization was transitory or that bacteria in the droppings were rapidly inactivated by dehydration and ultraviolet light.

Season, size, and extent of mobility of groups of Canada Geese, and vicinity to other sources of Campylobacter may account for the difference in prevalence observed between ours and previous studies (Fallacara et al., 2001). Furthermore, the cross-sectional nature of the study allowed us to survey a number of different groups of geese at a variety of sites but prevented us from assessing the temporal persistence of colonization status or of strain types. Our results indicate that for several sites, data from one year could not predict prevalence or strain types for the following year. However, for the two sites that were C. jejuni–positive in 2008 and 2009, isolates from Residential 1 in 2008 were highly similar to some of the isolates from the same site in 2009. The limited difference (the size of the largest SmaI fragment) could reflect genetic differentiation as the bacteria amplified in the geese.

Thermophilic campylobacters were not isolated from several sites, including four of those surveyed in 2008 and 2009. The reasons for C. jejuni being isolated from birds at some sites but not others are not clear and may reflect attributes of the feeding grounds at those sites or the extent of commingling with other individuals. Longitudinal studies are needed to assess the duration of C. jejuni–positive or C. jejuni–negative status in a given population.

Similar to previous reports, all campylobacter isolates were C. jejuni and were susceptible to a panel of antibiotics (Fallacara et al., 2001, 2004). A study of river water and waterfowl in Canada revealed that C. jejuni was the most frequently isolated Campylobacter species and also described recovery of Campylobacter lari from fecal samples of Canada Geese (Van Dyke et al., 2010). The isolation methods we employed were optimized for the recovery of thermophilic campylobacters (C. jejuni, C. coli, C. lari);
therefore, we were unable to exclude the presence of other *Campylobacter* species. Furthermore, prevalence data were based on direct plating on selective media, and higher prevalence may have been detected had selective enrichments been used. The choice for direct plating was made to allow unbiased strain recovery, as certain strains may outcompete others during selective enrichment protocols (Harder and Dijkhuizen, 1982; Dunbar et al., 1997).

The * fla-PFGE and MLST data indicated that a diverse collection of strains colonized the Canada Geese surveyed in this study. The sharing of water sources and adjacent land by multiple groups of resident Canada Geese would be expected to promote transmission of *Campylobacter* among geese and may account for ST-4080 becoming disseminated among several sites in 2009. The resident Canada Geese sampled moved freely among the sites and bodies of water in the area (M. E. Rutledge, unpubl. data), and *Campylobacter* may spread from one group of Canada Geese to individuals in other locations as birds move between foraging and roosting sites (Kassa et al., 2001). However, our study shows strain homogeneity within each sampled group, which may support a lack of mixing among the geese or with other avian sources of *Campylobacter*.

With the exception of one sequence type (ST-4071, clonal complex ST-1034), which was detected in *C. jejuni* from a case of human campylobacteriosis in Canada, the strain types of *C. jejuni* from the Canada Geese were not previously encountered among human clinical cases or among *C. jejuni* from food animals. The predominant sequence types, ST-4080 and ST-702, had been previously identified among *C. jejuni* from Canada Geese, while ST-991 (clonal complex ST-692) had been previously identified in *C. jejuni* from environmental water. Isolates of *C. jejuni* from water and wildlife appear to comprise a distinct clade with limited representation among human isolates (Hepworth et al., 2011). However, testing of two *C. jejuni* strains from Canada Geese in a day-old chick colonization model indicated that both were capable of colonizing the chicks (R. M. Siletzky and S. Kathariou, unpubl.), suggesting the potential of *C. jejuni* from Canada Geese to enter the poultry production system.

Due to differences in STs between resident Canada Geese and humans and the lack of antimicrobial resistance of the goose isolates, our study indicates that resident Canada Geese may not be a substantial source of *C. jejuni* infection in humans. More research is needed to assess the hazards of sharing locations with Canada Geese, including surveillance of human and animal samples from urban and animal production environments. Our objective and focus was to characterize *Campylobacter* in resident Canada Geese. However, *Salmonella* sampling (*n*=100) was also conducted in 2009, but no isolates were detected. The absence of *Salmonella* is similar to results from previous studies (Hussong et al., 1979; Fallacara et al., 2001; Wahlström et al., 2003).

**ACKNOWLEDGMENTS**

The project was funded in part by the Department of Forestry and Environmental Resources and the Fisheries, Wildlife, and Conservation Biology Program, and the Department of Food, Bioprocessing, and Nutrition Sciences at North Carolina State University, The Federal Aviation Administration, the US Department of Agriculture (USDA), Animal and Plant Health Inspection Service, Wildlife Services, the Berryman Institute, and USDA National Research Initiative Competitive Grant 2008-35201-04664. We thank the following individuals from North Carolina State University, College of Veterinary Medicine: P. Jay, E. Gebhard, C. Meek, K. Baine, E. Bradshaw, C. Shultz, and J. Gjeltema, for assistance with the field study, and S. Allen from the Fisheries, Wildlife, and Conservation Biology Program.

**LITERATURE CITED**


Submitted for publication 4 October 2011.

Accepted 18 June 2012.