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PREVALENCE OF THREE *CAMPYLOBACTER* SPECIES, *C. JEJUNI*, *C. COLI*, AND *C. LARI*, USING MULTILOCUS SEQUENCE TYPING IN WILD BIRDS OF THE MID-ATLANTIC REGION, USA

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ABSTRACT: *Campylobacter jejuni* is responsible for the majority of bacterial foodborne gastroenteritis in the US, usually due to the consumption of undercooked poultry. Research on which avian species transmit the bacterium is limited, especially in the US. We sampled wild birds in three families—Anatidae, Scolopacidae, and Laridae—in eastern North America to determine the prevalence and specific strains of *Campylobacter*. The overall prevalence of *Campylobacter* spp. was 9.2% for all wild birds sampled ($n=781$). *Campylobacter jejuni* was the most prevalent species (8.1%), while *Campylobacter coli* and *Campylobacter lari* prevalence estimates were low (1.4% and 0.3%, respectively). We used multilocus sequence typing PCR specific to *C. jejuni* to characterize clonal complexes and sequence types isolated from wild bird samples and detected 13 novel sequence types, along with a clonal complex previously only associated with human disease (ST-658). Wild birds share an increasing amount of habitat with humans as more landscapes become fragmented and developed for human needs. Wild birds are and will remain an important aspect of public health due to their ability to carry and disperse emerging zoonotic pathogens or their arthropod vectors. As basic information such as prevalence is limited or lacking from a majority of wild birds in the US, this study provides further insight into *Campylobacter* epidemiology, host preference, and strain characterization of *C. jejuni*.

Key words: Anatidae, *Campylobacter*, *Campylobacter jejuni*, Laridae, Mid-Atlantic region, Scolopacidae, wild birds.

INTRODUCTION

Three species of *Campylobacter*, *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari*, are of greatest concern to the poultry industry (Evans and Powell 2008). All three species can be isolated from poultry, but the most pathogenic in terms of human disease is *C. jejuni* (Evans and Powell 2008). *Campylobacter jejuni* is responsible for the majority of bacterial foodborne gastroenteritis in the US (Abulreesh et al. 2006), and infections have been linked to increased risk for Crohn's disease, ulcerative colitis, and other conditions such as reactive arthritis (Hill et al. 2003; Kahng 2009; Nielsen et al. 2009; Schonberg-Norio et al. 2009).

Campylobacter bacteria can exist in the avian gut as commensal organisms and are a component of the natural microbiota of some bird species (Joens 2004). In mature poultry, ducks, and turkeys, *Campylobacter* infections are nonpathogenic and generally asymptomatic (Wright et al. 2008), making detection of the bacterium challenging and therefore posing a potential threat to consumers (Newell and Fearnley 2003). In the US, little research has been conducted on wild avian species that have the potential for transmitting *Campylobacter* spp., yet this could be an important pathway for transmission. Keller et al. (2011) evaluated the prevalence of *Campylobacter* spp. in a subset of wild bird species, including herons, waterfowl, raptors, gulls, and various songbirds and found that *C. jejuni* prevalence was greatest in the Corvidae and Laridae. Yogasundram et al. (1989) estimated *C. jejuni* prevalence in 13 orders of wild birds in the US and found that the Psittaciformes, Galliformes, and Anseriformes had the highest prevalence. In Japan, Ito et al. (1988) found high *C. jejuni* prevalence in Japanese crows (34%), with an overall prevalence of 14% in all sampled bird species. Waldenström et al.

(2002) found *Campylobacter* in 21.6% of migrating birds in various taxa, while Ramos et al. (2010) isolated *Campylobacter* from 10% of sampled gull chicks. The vast majority of these studies occurred outside the US, further supporting the need to establish *Campylobacter* spp. prevalence in wild birds in the US.

We determined the prevalence of *Campylobacter jejuni*, *C. coli*, and *C. lari* in wild birds (Anatidae [waterfowl], Laridae [gulls], and Scolopacidae [shorebirds]) to identify DNA sequence similarities and differences in clonal complexes (CCs) and sequence types (STs) through multilocus sequence typing polymerase chain reaction (MLST-PCR). Information from this study contributes to the expansion of the global *Campylobacter* isolate database developed by Keith Jolley, sited at the University of Oxford (Jolley and Maiden 2010), giving further insight into epidemiology and how it relates to specific species of wild birds. Gulls are efficient carriers of zoonotic pathogens because they are attracted to altered human environments, including garbage dumps, untreated sewage, and manure (Reed et al. 2003). Canada Geese (*Branta canadensis*), and Snow Geese (*Chen caerulescens*) can also be found in proximity to human habitat, as well as on agricultural fields, predisposing them for transmission of zoonotic pathogens to humans. Shorebirds can carry high *Campylobacter* loads (79.6%) in Europe (Waldenström et al. 2002), but nothing is known about the prevalence of *Campylobacter* in these birds in North America.

MATERIALS AND METHODS

Sample collection

We collected wild bird fecal and cloacal swab samples at 26 sites throughout Delaware, Pennsylvania, and New Jersey from July 2009 to July 2011, using standardized procedures similar to those of Keller et al. (2011). We identified large, single-species flocks to sample adult geese and gulls and observed them prior to flushing to ensure we collected fresh fecal samples. We used cloacal swabbing to collect samples from hatch-year geese, gulls, and shorebirds while the birds were being handled by New Jersey Fish and Wildlife Services (NJ FWS) officials or the Delaware Shorebird Project, respectively.

We collected cloacal or fecal samples from Snow Geese, Canada Geese, Ring-billed Gulls (*Larus delawarensis*), Herring Gulls (*Larus argentatus*), Greater Black-backed Gulls (*Larus marinus*), Laughing Gulls (*Leucophaeus atricilla*), Ruddy Turnstones (*Arenaria interpres*), Red Knots (*Calidris canutus*), Sanderling (*Calidris alba*), and Semipalmated Sandpipers (*Calidris pusilla*). We collected all Snow Goose samples from one location in Pennsylvania on several sampling occasions during late winter (February–March). We collected Canada Goose samples from flocks in agriculture fields and ponds near human habitat, as well as during banding drives during the summer (June) with NJ FWS. We collected adult gull samples from urbanized habitats such as parking lots and from beaches. We collected hatch-year gull samples with NJ FWS during the summer (July) on several sampling occasions. We analyzed gull species collectively given the number of samples per species (Table 1).

We collected all samples with sterile standard cotton swabs and immediately placed them in 1.5-mL screw-cap microcentrifuge tubes containing a specialized medium (2x LB containing tryptone, NaCl, and yeast extract, with 2x salt containing K_2HPO_4, KH_2PO_4, Na-citrate, $MgSO_4 \times 7H_2O$, $[NH_4]_2SO_4$, with 87% glycerol; Gergen et al. 1979). We stored the microcentrifuge tubes containing the swabs on ice (4–7 C) until transferred to a −70 C freezer. For each sample, we assigned an identification number, a record of age or sex if known, and date and location the sample was taken.

Culture isolation

We cultivated samples by vortexing and plating on *Campylobacter*-selective blood-free charcoal-based agar plates supplemented with cefoperazone and amphotericin B (Oxoid, Manchester, Connecticut, USA). Plates were incubated 48 hr at 42 C in a microaerobic environment (85% N, 10% CO_2, 5% O_2) using CampyGen Packs (Oxoid) and BBL Campy EZ Gas Paks (BD, Sparks, Maryland, USA) in AnaeroJars (Oxoid) and GasPak Anaerobic Systems Jars (BD). Bacteria that showed characteristic *Campylobacter* colony growth, were positive for catalase and oxidase reactions, and could not grow in oxygen-rich environments were observed under the light microscope. Gram-negative curved rods were

TABLE 1. Wild bird fecal samples collected in Delaware (DE), Pennsylvania (PA), and New Jersey (NJ), USA, June 2009–June 2011.

Order	Family	Genus and species	County, state	No. samples
Anseriformes	Anatidae	*Chen caerulescens*	Lebanon, PA	111
		Branta canadensis	Salem, NJ	95
			Cumberland, NJ	89
			New Castle, DE	37
			Cape May, NJ	24
			Berks, PA	15
			Kent, DE	14
Charadriiformes	Scolopacidae	*Arenaria interpres*	Kent, DE	114
			Sussex, DE	10
		Calidris canutus	Kent, DE	57
		Calidris alba	Sussex, DE	26
		Calidris pusilla	Sussex, DE	37
			Kent, DE	25
	Laridae	*Larus delawarensis*	Kent, DE	9
		Larus argentatus	Cape May, NJ	47
			Kent, DE	19
		Larus marinus	Cape May, NJ	6
			Sussex, DE	1
		Leucophaeus atricilla	Cape May, NJ	43
			Sussex, DE	2

preliminarily considered to be *Campylobacter* species and were further isolated on 5% sheep blood tryptic soy agar (TSA) plates incubated microaerobically at 37 C for 24–48 hr. Isolates from these plates were frozen at −70 C in the collection medium.

DNA extraction and PCR

We thawed culture isolated samples and grew them on 5% sheep blood TSA plates. We prepared overnight cultures by scraping the 5% sheep blood TSA plates into a test tube containing 2 mL of Mueller-Hinton broth (BD) and incubating overnight at 42 C. DNA was extracted from bacterial cells using the Qiagen Gentra Puregene Yeast/Bacterial Kit for Gram-negative bacteria (Qiagen, Germantown, Maryland, USA). Following DNA extraction, samples were stored at −70 C.

We diluted extracted DNA to approximately 25 ng/μL and used multiplex PCR (MX-PCR; Vandamme et al. 1997) for the initial discrimination of *C. jejuni* and *C. coli*. We used 25-μL reactions using 1 μL of template DNA (~25 ng/μL), 5 μL of 5X Q solution, 2.5 μL of $MgCl_2$ (25 mM), and 2.5 μL of 10X PCR buffer containing 15 mM $MgCl_2$, 1 μL of dNTPs (10 mM), 1 μL each of the *C. coli* and *C. jejuni* forward and reverse primers (10 μM), 0.1 μL of Taq DNA polymerase (5 U/μL), and 8.9 μL of double-distilled (dd) H_2O for each reaction. All reagents used were from the Qiagen Taq PCR core kit, and the initial denaturization step was 1 min at 94 C. We used a PCR developed by Lawson et al. (1998) for detection of *C. lari* on samples that did not show appropriate bands at 350 bp for *C. coli*. We used 25-μL reactions using 1 μL of template DNA (~25 ng/μL), 5 μL of 5X Q solution, 2.5 μL $MgCl_2$ (25 mM), and 2.5 μL of 10X PCR buffer containing 15 mM $MgCl_2$, 0.5 μL of dNTPs (10 mM), 2 μL each of the *C. lari* forward and reverse primer (10 μM), 0.1 μL of Taq DNA polymerase (5 U/μL), and 9.4 μL of dd H_2O for each reaction. All reagents used were from the Qiagen Taq PCR core kit, and the initial denaturization step was 1 min at 94 C. We ran 1–2% agarose gels to visualize PCR products.

We used MLST PCR for *C. jejuni* on the samples that were negative for both the *C. coli* or *C. lari* PCRs or were positive for *C. jejuni* with the MX-PCR. For *C. jejuni*, the MLST scheme uses DNA sequences of internal fragments of seven housekeeping genes (Dingle et al. 2001). We used protocols (including primers) from the *Campylobacter* Multi Locus Sequence Typing website (Jolley and Maiden 2010). Reaction conditions were that of denaturization for 1 min at 94 C, annealing for 1 min at 50 C, and extension for 1 min at 72 C. This was followed by a final extension at 72 C for 10 min. We ran 25-μL reactions using 1 μL of template DNA (~25 ng/μL), 5 μL of 5X Q solution, and 2.5 μL of 10X PCR buffer

TABLE 2. Wild bird samples culture positive for *Campylobacter* spp., collected in Delaware, Pennsylvania, and New Jersey, USA, June 2009–June 2011.

Order	Family	Genus and species	HY[a]	AHY[b]	U[c]	Percentage (number) positive for *Campylobacter* growth
Anseriformes	Anatidae	*Chen caerulescens*		111		29.7 (33)
		Branta canadensis	36			17 (6)
				206		2.4 (5)
					32	19 (6)
		All geese	36	317	32	13.0 (50)
Charadriiformes	Scolopacidae	*Arenaria interpres*		124		33.9 (42)
		Calidris canutus		57		11 (6)
		Calidris alba		26		4 (1)
		Calidris pusilla		62		26 (16)
		All shorebirds		269		24.2 (65)
	Laridae	*Larus delawarensis*		9		22 (2)
		Larus argentatus	44			18 (8)
				22		14 (3)
		Larus marinus	6			0 (0)
				1		0 (0)
		Leucophaeus atricilla	42			2 (1)
				3		0 (0)
		All gulls	92	35		11.8 (15)

[a] HY = hatch year.

[b] AHY = after-hatch-year.

[c] U = unknown.

containing 15 mM $MgCl_2$, 0.5 μL of dNTPs (10 mM), 2 μL each of the designated housekeeping gene's forward and reverse primer (10 μM), 0.1 μL of Platinum Taq (Invitrogen, Carlsbad, California, USA) (5 U/μL), and 11.9 μL of dd H_2O for each reaction. Starred primers as indicated by the database usually give the best results and were used initially (Jolley and Maiden 2010). If a sample was not positive, the alternatively listed primers were used for each of the housekeeping genes. We visualized PCR products with 1–2% agarose gels.

PCR purification and sequencing

We purified samples for which the PCR product was positive for all seven housekeeping genes using a QiaQuick PCR purification system employing spin-columns (Qiagen). Between 7 and 7.5 μL of PCR product was used. Following PCR purification, we stored samples at −70 C. Purified samples and sequencing primers were sent to the University of Pennsylvania School of Medicine (Philadelphia, Pennsylvania, USA) DNA Sequencing Facility for DNA sequencing. Samples were run with the starred and double-starred primers, which usually give the best results, or have been shown to be more efficient with wild bird samples. If a sample did not amplify, the alternatively listed primers were used for each of the housekeeping genes as needed. For each sample, seven sequencing reactions were performed, each corresponding to one of the seven housekeeping genes. A distinct allele was assigned for each housekeeping gene. The most recent database query was performed on 20 February 2012.

RESULTS

We detected *Campylobacter* spp. in 17% of wild bird samples (n=781) through culturing methods (Table 2). Using PCR-based methods, all species except Greater Black-backed Gulls were positive for *Campylobacter*. Prevalence of *C. coli* and *C. lari* was low in these wild bird samples (0.9% and 0.3%, n=781). Snow Geese had the greatest prevalence of *C. coli* (6.3%, n=111); Herring Gulls were the only species to carry *C. lari* (3%, n=66).

Across all samples, 8.1% (n=781) were positive for *C. jejuni*, and 10.6% amplified at all seven *C. jejuni* housekeeping genes but did not provide full sequences at all

seven loci. We detected 17% *C. jejuni* prevalence in Snow Geese ($n=111$), amplifying at all seven housekeeping genes for the *C. jejuni* MLST scheme. All except two Snow Goose samples gave complete ST profiles when sequenced. We detected a 4.7% *C. jejuni* prevalence in Canada Geese ($n=274$), and most samples gave complete ST profiles. Forty-six percent of the positive Canada Goose samples were isolated from hatch-year geese, 15% from adults, and 39% from Canada Geese of unknown age. We detected 6.3% *C. jejuni* prevalence in gulls ($n=127$), and all but one sample provided sequences for all seven housekeeping genes. Half of these positive samples were from hatch-year birds. We detected 25% *C. jejuni* prevalence in Ruddy Turnstones ($n=124$), and 61% of these samples provided complete ST profiles. We detected an 11% *C. jejuni* prevalence in Semipalmated Sandpipers ($n=62$), and five of the seven positive samples gave complete ST profiles (Table 3).

Seventy-eight percent of culture-positive *Campylobacter* spp. samples were positive for *C. jejuni* by MX-PCR, while full STs were obtained from 76% of positive *C. jejuni* samples. Of these, 34 were assigned a clonal complex, and 63 were at least assigned a ST, 29 of which had an unknown CC. More than one third of the samples ($n=25$) did not yet have a ST assigned and were sorted into 13 novel STs by the curator of the *Campylobacter* Multi Locus Sequence Typing website (Table 3).

The most common CC was the ST-1287 complex, followed by the ST-1275 and ST-1034 complexes. Two Snow Goose samples were isolated from the ST-45 complex, and one Snow Goose sample was isolated from the ST-658 complex. For samples where no CC is currently assigned, ST-5705 was the most common, followed by ST-710. Sequence types 691, ST 693, ST 2514, and ST 3961 each occurred in two samples. Sequence types 2349, ST 3112, and ST 4535 each occurred in one sample. Sequence types 5879, ST 5880, ST 5881, ST 5883, ST 5889, and ST 5890 each occurred in one sample and had never been isolated before this study.

DISCUSSION

The overall prevalence of *Campylobacter* spp. for all wild birds in this study was 9.2%, similar to previously conducted studies investigating wild birds in the US (Yogasundram et al. 1989; Keller et al. 2011). Keller et al. (2011) estimated *C. jejuni* prevalence at 7.2% from six orders, while Yogasundram et al. (1989) estimated a 10.1% prevalence of *C. jejuni* from 13 orders of wild birds. In this study, samples were heterogeneous in terms of prevalence at all levels, including order, family, genus, and species, ranging from 0 to 43.1%. *Campylobacter jejuni* was the most prevalent species of *Campylobacter*, while *C. coli* and *C. lari* prevalence estimates were low (8.1%, 1.4%, 0.3%, respectively).

There was an uneven age distribution in samples analyzed among species, but also within species. Hatch-year Canada Geese and gulls had a higher prevalence of *Campylobacter* spp. than after-hatch-year birds. Dobbin et al. (2005) found *Campylobacter* in a subset of 2–3-wk-old Double-crested Cormorant chicks (*Phalacrocorax auritus*). Double-crested Cormorants nest in dense colonies similar to those of gulls, where bacteria may be easily shared. Hatch-year birds may come into contact with different bacteria such as *Campylobacter* as their immune system is developing, suggesting a higher prevalence in this age group.

Birds in the Anatidae had >27% prevalence in European studies (Waldenström 2005) and specifically 15% in Canada Geese in Europe (Wahlstrom et al. 2003). In contrast, migrating Graylag Geese (*Anser anser*) in the Netherlands had low *Campylobacter* prevalence (Lillehaug et al. 2005). In North America, Canada Geese harbor *C. jejuni* infrequently, as Pacha et al. (1988) and Keller et al. (2011) described (5% and 0%, respectively). Snow Geese, a

TABLE 3. Clonal complex (CC) and sequence type (ST) distribution for *Campylobacter jejuni* in wild bird samples analyzed through multilocus sequence typing (MLST) PCR collected in Delaware, Pennsylvania, and New Jersey, USA, June 2009–June 2011. Multiplex (MX)-PCR results also shown. An "x" indicates no sequence identified.

		MLST-PCR							MX-PCR		
CC	ST	aspA	glnA	gltA	glyA	pgm	tkt	uncA	*C. jejuni*	Sample ID	Source
ST-45	45	4	7	10	4	1	7	1	−	91717	*Chen caerulescens*
		4	7	10	4	1	7	1	+	91718	*Chen caerulescens*
ST-658	658	2	4	2	4	19	3	6	+	91741	*Chen caerulescens*
ST-1034	1033	2	61	4	64	126	7	23	+	91714	*Chen caerulescens*
	2525	2	15	4	27	126	25	23	+	91756	*Chen caerulescens*
	4106	2	106	4	48	470	25	23	+	91629	*Branta canadensis*
	5882	2	61	4	64	126	136	23	+	91761	*Chen caerulescens*[c]
ST-1275	637	10	33	22	49	43	82	31	−	91980	*Larus delawarensis*
	1223	27	33	22	49	43	9	31	+	91974	*Larus argentatus*
	1268	27	33	22	49	43	20	31	+	91938	*Larus argentatus*
		27	33	22	49	43	20	31	+	92005	*Larus argentatus*
		27	33	22	49	43	20	31	+	92012	*Larus argentatus*
	1275	27	33	22	49	43	82	31	+	91985	*Larus delawarensis*
ST-1287	1287	84	106	29	28	74	136	90	−	91899	*Calidris pusilla*
		84	106	29	28	74	136	90	+	91910	*Arenaria interpres*
	1305	84	106	29	154	74	136	90	−	92387	*Calidris canutus*
	1328	84	148	29	28	74	136	90	−	92326	*Calidris canutus*
		84	148	29	28	74	136	90	+	92287	*Arenaria interpres*
	1352	84	141	29	144	74	183	90	+	91920	*Arenaria interpres*
		84	141	29	144	74	183	90	+	92307	*Calidris canutus*
	3039	84	141	29	28	74	136	232	−	91883	*Arenaria interpres*
	5884	84	106	29	28	74	25	90	−	91870	*Arenaria interpres*[c]
		84	106	29	28	74	25	90	−	92397	*Calidris pusilla*[c]
	5885	84	106	29	28	74	29	87	+	91903A	*Arenaria interpres*[c]
	5886	84	106	29	28	74	136	87	+	91872	*Arenaria interpres*[c]
		84	106	29	28	74	136	87	+	91881	*Arenaria interpres*[c]
		84	106	29	28	74	136	87	+	92322	*Calidris canutus*[c]
		84	106	29	28	74	136	87	+	92300	*Arenaria interpres*[c]
		84	106	29	28	74	136	87	+	92345	*Arenaria interpres*[c]
	5887	84	106	29	28	74	388	90	−	92406	*Calidris pusilla*[c]
	5888	84	106	29	28	526	136	116	−	91948	*Calidris pusilla*[c]
		84	106	29	28	526	136	116	−	91924	*Arenaria interpres*[c]
	6079	84	106	29	28	74	136	89	−	92273	*Arenaria interpres*[c]
	6080	84	106	115	28	74	161	87	+	92373	*Arenaria interpres*[c]
U[a]	691	2	59	4	27	126	24	23	+	91784	*Chen caerulescens*
		2	59	4	27	126	24	23	+	91790	*Chen caerulescens*
	693	2	29	4	48	13	24	57	+	91713	*Chen caerulescens*
		2	29	4	48	13	24	57	+	91740	*Chen caerulescens*
	710	37	29	75	48	126	25	23	+	91743	*Chen caerulescens*
		37	29	75	48	126	25	23	+	91745	*Chen caerulescens*
		37	29	75	48	126	25	23	+	91763	*Chen caerulescens*
	2349	2	59	4	48	131	24	57	+	91618	*Branta canadensis*
	2514	2	59	4	48	13	24	57	+	91560	*Branta canadensis*
		2	59	4	48	13	24	57	+	91568	*Branta canadensis*
	3112	2	4	4	48	13	25	57	+	92099	*Branta canadensis*
	3961	2	29	4	27	10	25	24	+	91622	*Branta canadensis*
		2	29	4	27	10	25	24	+	91623	*Branta canadensis*
	4535	10	7	269	4	261	3	50	−	91693	*Chen caerulescens*
	5705	2	4	84	459	126	25	57	+	92183	*Branta canadensis*
	5705	2	4	84	459	126	25	57	+	92186	*Branta canadensis*
	5705	2	4	84	459	126	25	57	+	92188	*Branta canadensis*
		2	4	84	459	126	25	57	+	92217	*Branta canadensis*

TABLE 3. Continued.

		MLST-PCR							MX-PCR		
CC	ST	aspA	glnA	gltA	glyA	pgm	tkt	uncA	*C. jejuni*	Sample ID	Source
	5879	2	29	4	48	10	25	24	+	91770	*Chen caerulescens*[c]
	5880	2	29	4	48	13	3	57	+	91777	*Chen caerulescens*[c]
	5881	2	59	4	48	131	25	238	+	91735	*Chen caerulescens*[c]
	5883	27	7	31	18	178	136	16	+	91950	*Larus delawarensis*[c]
	5889	84	121	20	144	74	183	90	+	91921	*Arenaria interpres*[c]
	5890	84	141	29	144	388	136	87	+	91856	*Arenaria interpres*[c]
		84	141	29	144	388	136	87	+	91878	*Arenaria interpres*[c]
		84	141	29	144	388	136	87	+	91884	*Arenaria interpres*[c]
		84	141	29	144	388	136	87	+	91926	*Arenaria interpres*[c]
		84	141	29	144	388	136	87	+	91946	*Calidris pusilla*[c]
		84	141	29	144	388	136	87	+	92372	*Arenaria interpres*[c]
P[b]		2	4	4	48	13	161	x	+	91767	*Chen caerulescens*
		2	4	84	459	126	25	x	+	92181	*Branta canadensis*
		2	403	4	105	126	25	x	+	91715	*Chen caerulescens*
		27	33	22	49	43	9	x	−	92007	*Larus argentatus*
		37	52	57	26	129	29	x	+	92187	*Branta canadensis*
		84	106	115	28	74	161	x	+	91871	*Arenaria interpres*
		84	141	29	28	526	20	x	+	92417	*Calidris pusilla*
		84	141	29	144	388	136	x	+	91875	*Arenaria interpres*
		84	141	29	x	x	161	87	+	92328	*Calidris canutus*
		84	x	29	x	74	183	90	+	92348	*Arenaria interpres*
		84	x	29	144	x	183	87	+	92285	*Arenaria interpres*
		84	x	29	x	74	136	87	+	92299	*Arenaria interpres*
		84	x	29	144	x	183	87	+	92346	*Arenaria interpres*
		206	x	29	144	388	25	87	+	91851	*Arenaria interpres*
		206	x	29	144	388	161	231	+	91923	*Arenaria interpres*
		84	x	29	x	x	161	x	+	92366	*Arenaria interpres*
		84	x	x	x	x	136	x	−	91925	*Arenaria interpres*
		x	33	x	x	x	x	x	−	91877B	*Arenaria interpres*
		x	x	x	x	x	25	x	−	91906B	*Arenaria interpres*
		x	x	x	x	x	161	x	−	91890	*Calidris pusilla*

[a] Unassigned clonal complex.

[b] Partial sequence type.

[c] Novel sequence type identified in this study.

migratory goose species, had the highest *C. jejuni* prevalence rate of geese in our study, while the resident Canada Geese samples had a much lower prevalence rate. Seasonal variation of *Campylobacter* abundance could have played a role in these differences (Taylor et al. 2013). Besides varying prevalence among species due to migration status, temporal components such as seasonal variation in *Campylobacter* abundance could also have played a role in the patterns we detected (Broman et al. 2002; Taylor et al. 2013). We sampled Snow Geese in winter and Canada Geese throughout the year, with the majority of Canada Goose samples being collected during the summer.

All shorebird species sampled in this study were migratory. Ruddy Turnstones undergo long-distance complete migrations with no resident populations (Harrington and Flowers 1996), and they had the highest prevalence of *C. jejuni* (15.3%, $n=124$). Hughes et al. (2009) found that European Ruddy Turnstones had a 0% ($n=27$) prevalence. This may be because these birds are a different subspecies and have a different migration route. Like migratory Snow Geese, migratory shorebird species tended to have a higher

Campylobacter prevalence than nonmigratory or less migratory wild bird species in this study. In addition, small flocks of Ruddy Turnstones often intermingle with flocks of Red Knots (*Calidris canutus*), Semipalmated Sandpipers, and Sanderling (Harrington and Flowers 1996). This interspecific flocking behavior offers opportunities for bacterial exchange on stopover sites such as the Delaware Bay, where these samples were taken. Semipalmated Sandpipers and Red Knots had the second highest prevalences of *C. jejuni* (8.1% and 7%, respectively). Semipalmated Sandpipers also undergo long-distance migrations between North and South America, with a limited number of stopover sites (Rodrigues 2000). Red Knots, one of the longest-distance migrating birds, make prolonged stops at migration staging sites to prepare for extreme long-distance flights (Piersma 1987). Differences in prevalence may relate to migration route, frequency and use of stopover sites, feeding behavior, ability to encounter the pathogen, species physiology, and exposure rate. We did not detect *Campylobacter* species in Sanderling, even though these birds share the same stopover points and occur in proximity to other species. There is a large range in Sanderling migration distances and durations as compared to the other shorebird species sampled (Myers et al. 1985). Opposite effects have been shown in songbirds (Passeriformes), where *Campylobacter* prevalence was higher in short-distance migrants (17.2%) and lower in long-distance migrants (31.5%) (Sensale et al. 2006). This may be attributed to differences in physiology, as shorebird migrations tend to be much more strenuous for individuals with fewer stopover points. Therefore, higher stress during the migration event could cause greater susceptibility to opportunistic commensal organisms. Greater prevalence of *Campylobacter* in shorebirds may also indicate a strong commensal relationship with the bacteria (Hughes et al. 2009).

There was a large variety of STs and CCs present from the two orders and three families of wild birds in this study, indicating that wild birds potentially harbor a wide array of pathogenic *Campylobacter*. Overall 5 CCs and 37 assigned STs, 13 of which were previously unassigned, were isolated. The majority of CCs we isolated had other wild bird or environmental water samples associated with them. It is generally thought that *C. jejuni* isolates that had only been isolated from wild birds may not be involved in human disease (Hughes et al. 2009). However, as isolates have been added to the database, human disease has been associated with complexes thought to only be associated with wild birds (Hughes et al. 2009; Sheppard et al. 2011). The ST-45 complex and the ST-658 complex, isolated from Snow Geese, are characteristic CCs associated with human disease. The ST-658 complex has only been isolated from human stool in Europe. As sample size increases in the database, wild bird samples are also being added to these complexes, also suggesting that wild birds are exposed to some strains thought to be exclusive to humans. Lu et al. (2009) argued that waterfowl can amplify and possibly transmit pathogens to humans by directly contaminating agricultural fields or surface waters. However, this process works both ways. Agricultural fields contaminated with untreated manure, for example, can directly infect wildlife. The ST-45 complex currently contains 464 isolates, approximately 55% of which have been isolated from human disease incidents. Two Snow Goose samples were isolated from this CC, which has been isolated from Macaroni Penguins (*Eudyptes chrysolophus*), even though *C. jejuni* persistence is rare among Antarctic animals (Griekspoor et al. 2009). This suggests that the strain could have been introduced through human disturbance. This supports the assertion that humans and poultry operations are contaminating wildlife with some human-dominated *Campylobacter* lineages.

The ST-1287 complex, which included *Campylobacter* from Ruddy Turnstones,

Red Knots, and Semipalmated Sandpipers, had previously been isolated from other shorebirds throughout the world, such as Dunlin (*Calidris alpina*) and Sharp-tailed Sandpipers (*Calidris acuminata*). The same scheme holds for the ST-1275 complex, mainly isolated from gulls (Black-headed Gulls [*Chroicocephalus ridibundus*]), and the ST-1034 complex, isolated from geese. In our study, the ST-1034 complex was isolated from Snow Geese and Canada Geese, while it had previously been isolated mostly from unnamed goose species and a Snow Goose in Canada. This suggests a tight taxonomic relationship between specific *C. jejuni* clonal complexes and their preferred host, possibly even coevolution of commensal strains.

Thirteen STs, comprising 25 samples or 39.7% of all STs, were novel STs not previously isolated. This is similar to studies evaluating *C. jejuni* strains in wild birds (Colles et al. 2008; French et al. 2009; Hughes et al. 2009). The Snow Goose and Canada Goose samples amplified well with the developed PCR and sequencing primers, while amplifying and obtaining usable DNA sequences from these samples was more challenging. Several species of geese have domesticated varieties. Therefore, *Campylobacter* spp. typing may be more efficient with goose samples (Griekspoor et al. 2013). Like gulls, geese tend to come into more contact with humans and have the potential to pick up human-specific strains more frequently. Overall, however, *Campylobacter* prevalence in gulls was low and confined to a single CC (ST-1275), suggesting that, at least in terms of spreading the enteropathogen *Campylobacter*, after-hatch-year gulls are of less concern than previously suggested.

Wild birds share an increasing amount of habitat with humans as landscapes become fragmented and developed for human needs. Wild birds will remain important to public health because of their ability to carry emerging zoonotic pathogens or aid in the dispersal of arthropod vectors (Reed et al. 2003). Although wild birds have the potential to spread disease organisms such as *Campylobacter*, humans are also leaking pathogens into the environment that have the potential for infecting wild birds. Even though some groups of birds such as shorebirds tend to carry their own commensals and unique STs and CCs, ultimately finding human disease associated CCs in these birds could be used as an indicator for further spread of some pathogens into the environment. As basic information such as prevalence is still not available from a great majority of wild bird species in the US, our study provides further insight into *Campylobacter* epidemiology, host preference, and strain characterization of *C. jejuni*.

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