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Molecular Epidemiology and Characterization of *Campylobacter* spp. Isolated from Wild Bird Populations in Northern England▽

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Campylobacter **infections have been reported at prevalences ranging from 2 to 50% in a range of wild bird species, although there have been few studies that have investigated the molecular epidemiology of *Campylobacter* spp. Consequently, whether wild birds are a source of infection in humans or domestic livestock or are mainly recipients of domestic animal strains and whether separate cycles of infection occur remain unknown. To address these questions, serial cross-sectional surveys of wild bird populations in northern England were carried out over a 2-year period. Fecal samples were collected from 2,084 wild bird individuals and screened for the presence of *Campylobacter* spp. A total of 56 isolates were recovered from 29 birds sampled at 15 of 167 diverse locales. *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* were detected by PCR, and the prevalences of different *Campylobacter* spp. in different avian families ranged from 0% to 33%. Characterization of 36 *C. jejuni* isolates by multilocus sequence typing revealed that wild birds carry both livestock-associated and unique strains of *C. jejuni*. However, the apparent absence of unique wild bird strains of *C. jejuni* in livestock suggests that the direction of infection is predominantly from livestock to wild birds. *C. lari* was detected mainly in wild birds sampled in an estuarine or coastal habitat. Fifteen *C. lari* isolates were analyzed by macrorestriction pulsed-field gel electrophoresis, which revealed genetically diverse populations of *C. lari* in Eurasian oystercatchers (*Haematopus ostralegus*) and clonal populations in magpies (*Pica pica*).**

Infection with *Campylobacter* spp. continues to be the leading cause of human infectious intestinal disease in the United Kingdom and has a significant economic impact (39). Consequently, there is a continuing effort to identify effective control methods. The majority of human infections (~90%) are caused by *Campylobacter jejuni* subsp. *jejuni* (46). Other *Campylobacter* species, including *Campylobacter coli* and *Campylobacter lari*, can also cause enteritis in humans, but their prevalence is lower. Most *C. jejuni* infections are believed to result from consumption of contaminated food, including poultry meat (27, 40), red meat (52), and milk (13), which is thought to be contaminated primarily by feces. It is well established that most livestock species, including poultry, ruminants, and pigs, carry *C. jejuni* asymptomatically (27), making control at the farm level difficult. However, the epidemiology of *C. jejuni* cannot be explained solely by food-borne exposure; *C. jejuni* has also been isolated from a range of environmental samples, including samples of soil, water, sand, and the feces of a number of wildlife species, including wild birds (1–3). However, the role that non-food-borne exposure plays in the epidemiology of *C. jejuni* is currently not well defined.

High prevalences of *Campylobacter* species infections have been found in a wide range of wild bird species, although there is great variation between taxa (2, 4, 7, 16, 35, 47, 48). Given their ability to fly long distances and their ubiquity, wild birds have the potential to play an important role in the epidemiology and evolution of *Campylobacter* spp. However, whether wild birds are a source of infection for humans or domestic livestock or are mainly recipients of domestic animal strains or, indeed, whether separate cycles of infection occur remain unknown. These questions remain unanswered in part because investigations of the epidemiology of *Campylobacter* spp. have been complicated by their high inter- and intraspecies genetic diversity (6).

The methods that have been routinely used to characterize *Campylobacter* isolates are restricted due to genomic instability in *Campylobacter* populations (10, 38, 45). Multilocus sequence typing (MLST) is a method that has the advantage of being objective since it is sequence based, which allows comparison of isolates from different laboratories and accurate determination of relationships between isolates from diverse sources (11). MLST studies of *C. jejuni* in farm animals and the environment, including wildlife, suggest that some strains may be associated with particular host groups (6, 10, 15, 30). However, in the same studies other strains were found to occur in several host species or habitats. Few studies have investigated the molecular epidemiology of *Campylobacter* infection in wild bird populations using MLST, and because only a relatively small number of isolates from wild

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birds have been characterized by MLST, conclusions have not been drawn yet about how wild bird isolates fit into the overall phylogenetic scheme or whether wild birds act as reservoirs, amplifiers, or merely indicators of infection of domestic animals with zoonotic genotypes.

In the current study a large cross-sectional survey of wild bird populations in northern England was undertaken to investigate the epidemiology of *Campylobacter* infection. Previous studies that have focused on the epidemiology of *Campylobacter* spp. solely in wild birds have investigated either a narrow range of taxonomic groups (2, 5, 17, 23, 29, 33, 43, 50) or wild birds from a limited range of habitats (18, 25, 48). Studies that have investigated a broad range of wild bird species have used *Campylobacter* characterization techniques that do not allow conclusions about possible host associations to be drawn or comparison of the genetic diversity of isolates between studies (21, 25, 34, 47, 53). Therefore, the aims of this study were (i) to determine the host range and prevalence of *Campylobacter* spp. in a wild bird population and (ii) through molecular characterization of isolates to determine whether wild birds were a likely source of infection in humans or domestic livestock and whether separate cycles of infection with host-adapted strains of *Campylobacter* spp. were maintained in the wild bird population.

MATERIALS AND METHODS

Study design. Serial cross-sectional surveys of wild bird populations throughout northern England were performed between July 2004 and October 2006. Samples were collected from a broad range of wild bird species, including representatives of the majority of wild bird families that are present in the United Kingdom for part of the year (migratory species) or the entire year (resident species). Wild bird individuals were the basic sampling unit.

Sample sources. Samples were collected from both live and dead wild birds. All live wild birds sampled in this study were trapped in collaboration with licensed British Trust for Ornithology bird ringers (36), and birds were caught by a variety of methods, including mist netting, cannon netting, ground traps, wildfowl round-ups, sampling nestlings in the nest, and environmental sampling.

Dead wildfowl and corvids were provided by licensed shooters, and dead garden birds were collected as part of the Garden Bird Health Initiative, a study investigating causes of garden bird mortality (8).

Sample collection. Once captured, each live bird was placed in a paper bag (R. S. Mulvihill, and R. C. Leberman, Powdermill Banding Station protocol, 2006 [http://www.westol.com/~banding/PowdermillBandingProtocol_Jan2006b.pdf]), in which it usually defecated, allowing a fecal sample to be collected. A new paper bag was used for each bird. Fecal samples were collected from the bags with sterile cotton-tipped swabs, placed in bacterial transport medium (Medical Wire and Equipment Ltd., Bath, United Kingdom), and transported to the laboratory in a cool box. If the bird did not defecate in the bag or the bird was too large to place in a bag, a cloacal swab sample was obtained. Occasionally, fecal samples were collected from the environment with sterile swabs. Fecal samples were also collected from the lower intestine of dead birds during postmortem examination.

Data collection. Data for each bird and the site at which the bird had been caught or found were recorded in the field. The date, location, and type of habitat where the bird had been trapped or found dead were recorded along with the capture method, British Trust for Ornithology ring number, species, age, and sex (36). Depending on the species or time of year, not all of these data could be obtained.

Bacterial culture. *Campylobacter* spp. were isolated from fecal samples using *Campylobacter* enrichment broth (LabM, Bury, United Kingdom) containing 10% lysed horse blood and were cultured on modified charcoal-cefoperazone-deoxycholate agar (LabM). Up to four colonies showing typical morphological characteristics of *Campylobacter* spp. (gray or white, small, round [diameter, 1 to 3 mm] colonies that are flat often with a raised center on modified charcoal-cefoperazone-deoxycholate agar) were subcultured onto Columbia agar (LabM) supplemented with 5% defibrinated horse blood. Isolates that grew under microaerobic conditions and did not grow under aerobic conditions were identified as presumptive *Campylobacter* isolates and were frozen in Microbank tubes (Pro-Lab Diagnostics, Neston, United Kingdom) at −80°C until they were required.

Molecular assignment to *Campylobacter* species by PCR. DNA extracts were prepared for each isolate by boiling one bead from each Microbank tube in 0.5 ml of sterile distilled water for 20 min. Cell lysates were kept at 4°C for no longer than 14 days.

Isolates were confirmed to be *C. jejuni*, *C. coli*, or *C. lari* using a previously described multiplex PCR that targeted the *hipO* and 23S rRNA genes of *C. jejuni* and the *glyA* gene of both *C. coli* and *C. lari* (49).

Three further single-PCR assays were used to amplify the *ceuE* gene of both *C. jejuni* and *C. coli* (19) and the 16S rRNA gene of *C. lari* (28) to confirm the results generated by the multiplex PCR assay described above (49). A duplex PCR was used to detect the 16S rRNA gene of *Campylobacter fetus* (both *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*) and *Campylobacter hyointestinalis* (28).

MLST of *C. jejuni* isolates. Chromosomal DNA was extracted from freshly grown *C. jejuni* using a NucleoSpin tissue DNA extraction kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. Segments of seven housekeeping genes, *aspA* (aspartase), *glnA* (glutamine synthetase), *gltA* (citrate synthase), *glyA* (serine hydroxymethyl transferase), *pgm* (phosphoglucomutase), *tkt* (transketolase), and *uncA* (ATP synthase alpha subunit), were amplified by PCR and sequenced using protocols, primers, and reaction conditions described previously (11). Sequencing reactions were carried out using an ABI Prism 3130x genetic analyzer (Applied Biosystems, California).

The STADEN software package (44) was used to assemble sequences from the chromatograms generated by the ABI Prism 3130x genetic analyzer (Applied Biosystems), and allele numbers were assigned by comparing sequences with sequences in the public MLST profile database (http://pubmlst.org/campylobacter). Sequence types (STs) and clonal complexes were also assigned by comparison with the STs and clonal complexes in the MLST database. Novel sequences and STs were submitted to the database, and new allele and ST numbers were assigned.

MLST was not attempted with the *C. coli* isolates as three of the four PCR-positive *C. coli* isolates could not be revived from storage at −80°C.

Genetic analysis of *C. jejuni* isolates. The genotypic relatedness of *C. jejuni* isolates was investigated by construction of an unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on a distance matrix of pairwise differences in allelic profiles using the START (Sequence Type Assignment Recombination Tests) software package (22).

Relationships between isolates and identification of founder strains were explored using the burst algorithm (based on related STs) (14, 22) in the START software package. Isolates were grouped together if they shared five, six, or seven alleles. Allelic profiles of isolates from this study were compared with the allelic profiles of all *C. jejuni* isolates obtained from wild bird sources in the public MLST database (http://pubmlst.org/campylobacter). The population structure of the isolates collected as part of our study was then compared with the population structure of the larger unstructured data set using the eBURST program to obtain a "population snapshot" of the data set (14).

Linkage analysis using the standardized index of association (I^S_A) (20) was carried out using the LIAN (linkage analysis) program in the START package (22).

PFGE of *C. lari* isolates. Pulsed-field gel electrophoresis (PFGE) of all *C. lari* isolates was performed using a previously described protocol (37). The BioNumerics software (version 4.0; Applied Maths BVBA, Sint-Martens-Latem, Belgium) was used for image analysis. Levels of similarity between pulsed-field banding patterns were determined using the Dice similarity coefficient method with a 2% tolerance window, and a UPGMA dendrogram was constructed.

Urease production assay with *C. lari* isolates. To detect any urease-positive thermophilic strains of *C. lari*, all *C. lari* isolates were grown on urea agar containing 5% (vol/vol) urea (LabM), which was incubated for 48 h under microaerobic conditions. The urea agar contained a pH-sensitive indicator that was pink in the presence of the hydrolysis products of urea, which was indicative of urease production.

Statistical analysis. The prevalence of each *Campylobacter* sp. detected and the 95% confidence intervals (95% CI) were determined using the frequency command in the statistical software package EpiInfo 2002 (CDC, Atlanta, GA).

RESULTS

Bacterial isolates. Fecal samples were collected from 2,084 individual wild birds belonging to 99 species from 167 locations between July 2004 and October 2006. A total of 59 *Campy-*

TABLE 1. Number of individuals of each wild bird species that were positive for *Campylobacter* spp. as determined by PCR

Family	Common name	Latin name	n^a	No. of PCR-positive wild bird individuals (no. of isolates)			Location(s)[b]
				C. jejuni	*C. coli*	*C. lari*	
Anatidae	Mute swan	*Cygnus olor*	30	0	1 (2)	0	6
	Eurasian wigeon	*Anas penelope*	43	0	0	1 (1)	12
Falconidae	Common kestrel	*Falco tinnunculus*	14	0	1 (1)	0	6
Rallidae	Common moorhen	*Gallinula chloropus*	3	1 (3)	0	0	4
Hematopodidae	Eurasian oystercatcher	*Hematopus ostralegus*	95	0	0	6 (10)	13, 14
Scolopacidae	Red knot	*Calidris canutus*	14	0	1 (1)	0	14
	Ruddy turnstone	*Arenaria interpres*	27	0	0	1 (1)	15
Laridae	Black-headed gull	*Larus ridibundus*	13	0	0	1 (1)	4
Columbidae	Rock dove or feral pigeon	*Columba livia*	47	2 (7)	0	0	3, 5
Tytonidae	Barn owl	*Tyto alba*	24	1 (1)	0	0	8
Turdidae	Common blackbird	*Turdus merula*	53	1 (3)	0	0	1
Paridae	Great tit	*Parus major*	154	1 (1)	0	0	1
Corvidae	Magpie	*Pica pica*	71	2 (6)	0	3 (5)	7, 9, 12
	Eurasian jay	*Garrulus glandarius*	3	1 (1)	0	0	10
	Eurasian jackdaw	*Corvus monedula*	6	1 (3)	0	0	6
Sturnidae	Common starling	*Sturnus vulgaris*	9	1 (3)	0	0	4
Passeridae	House sparrow	*Passer domesticus*	86	1 (4)	0	0	2
Fringillidae	Chaffinch	*Fringilla coelebs*	84	1 (4)	0	0	11
	European greenfinch	*Carduelis chloris*	93	0	0	1 (1)	3
Total				13 (36)	3 (4)	13 (19)	

[a] Total number of individuals sampled.
[b] Location(s) at which birds were sampled or were found dead. Locations 1 to 4 and 6 to 11, mixed pasture and arable farmland on the Cheshire plain in northwest England; location 5, a small town in Cheshire in northwest England; location 12, salt marsh habitat in the Mersey estuary in northwest England; locations 13 and 15, Morecambe Bay estuary in northwest England; location 14, Dee estuary in northwest England.

lobacter species isolates were recovered from fecal samples from 29 wild bird individuals belonging to 19 species sampled in 15 different locations, giving an overall *Campylobacter* species prevalence of 1.4% (95% CI, 1.0 to 2.0%) (Table 1). Three *Campylobacter* species were detected, *C. jejuni* (prevalence, 0.6% [95% CI, 0.4 to 1.1%]), *C. lari* (prevalence, 0.6% [95% CI, 0.4 to 1.1%]), and *C. coli* (prevalence, 0.1% [95% CI, 0.05 to 0.4%]).

Thirty-six *C. jejuni* isolates were isolated from fecal samples from 13 individual wild birds belonging to 11 species, including the common moorhen (*Gallinula chloropus*), feral pigeon (*Columba livia*), barn owl (*Tyto alba*), common blackbird (*Turdus merula*), great tit (*Parus major*), magpie (*Pica pica*), Eurasian jay (*Garrulus glandarius*), Eurasian jackdaw (*Corvus monedula*), common starling (*Sturnus vulgaris*), house sparrow (*Passer domesticus*), and chaffinch (*Fringilla coelebs*).

Nineteen *C. lari* isolates were recovered from fecal samples from 13 wild bird individuals belonging to six species, including the Eurasian wigeon (*Anas penelope*), Eurasian oystercatcher (*Haematopus ostralegus*), ruddy turnstone (*Arenaria interpres*), black-headed gull (*Larus ridibundus*), magpie, and European greenfinch (*Carduelis chloris*).

Four *C. coli* isolates were isolated from fecal samples from three wild bird individuals belonging to three species, the mute swan (*Cygnus olor*), common kestrel (*Falco tinnunculus*), and red knot (*Calidris canutus*). On no occasion were individual wild birds infected with more than one species of *Campylobacter*. For all but one wild bird species, one species of *Campylobacter* was isolated from fecal samples; magpies were the only wild bird species from which both *C. jejuni* and *C. lari* were isolated (Table 1).

Diversity of MLST STs. By using MLST, a total of 15 *C. jejuni* STs were identified for the 36 *C. jejuni* isolates examined (Table 2). The 36 *C. jejuni* isolates were detected in samples collected from 13 wild bird individuals belonging to 11 species. There were only two wild bird species from which *C. jejuni* was isolated from more than one individual (two feral pigeons and two magpies). All isolates derived from different individuals of the same species had different STs. Sixty-seven different alleles were identified for the seven loci, three of which were novel.

TABLE 2. MLST allelic profiles and STs of 36 *C. jejuni* isolates detected in the fecal samples from 13 wild birds[a]

Clonal complex	ST	Allelic profile (allele no.)							Source[e]	Location[f]	Sample collection date (day/mo/yr)
		aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*			
48	ST66	2	4	5	2	7	1	5	Great tit 1	1	22/7/2004
	ST48	2	4	1	2	7	1	5	House sparrow 1	2	14/1/2005
		2	4	1	2	7	1	5	House sparrow 1	2	14/1/2005
		2	4	1	2	7	1	5	House sparrow 1	2	14/1/2005
		2	4	1	2	7	1	5	House sparrow 1	2	14/1/2005
179	ST3001[c]	1	6	29	40	40	32	3	Feral pigeon 1	3	28/1/2005
	ST220	1	6	29	2	40	32	3	Feral pigeon 1	3	28/1/2005
	ST3002[c]	1	6	29	289	40	32	3	Feral pigeon 1	3	28/1/2005
U[b]	ST3274[c]	18	33	22	326[d]	1	86	16	Blackbird 1	1	3/3/2005
		18	33	22	326[d]	1	86	16	Blackbird 1	1	3/3/2005
		18	33	22	326[d]	1	86	16	Blackbird 1	1	3/3/2005
45	ST45	4	7	10	4	1	7	1	Moorhen 1	4	11/3/2005
		4	7	10	4	1	7	1	Moorhen 1	4	11/3/2005
		4	7	10	4	1	7	1	Moorhen 1	4	11/3/2005
		4	7	10	4	1	7	1	Barn owl 1	8	6/6/2005
		4	7	10	4	1	7	1	Magpie 2	9	20/6/2005
		4	7	10	4	1	7	1	Magpie 2	9	20/6/2005
		4	7	10	4	1	7	1	Magpie 2	9	20/6/2005
42	ST42	1	2	3	4	5	9	3	Feral pigeon 2	5	16/3/2005
		1	2	3	4	5	9	3	Feral pigeon 2	5	16/3/2005
		1	2	3	4	5	9	3	Feral pigeon 2	5	16/3/2005
		1	2	3	4	5	9	3	Feral pigeon 2	5	16/3/2005
U	ST3003[c]	64	98	20	100	134	94	16	Jackdaw 1	6	26/4/2005
		64	98	20	100	134	94	16	Jackdaw 1	6	26/4/2005
U	ST3275[c]	64	288[d]	71	189	134	94	60	Jackdaw 1	6	26/4/2005
1275	ST1231	27	2	22	104	43	86	31	Magpie 1	7	9/5/2005
		27	2	22	104	43	86	31	Magpie 1	7	9/5/2006
		27	2	22	104	43	86	31	Magpie 1	7	9/5/2005
U	ST2303	18	243	208	18	345	86	47	Jay 1	10	1/12/2005
U	ST3276[c]	216[d]	22	22	98	116	101	16	Chaffinch 1	11	15/5/2006
		216[d]	22	22	98	116	101	16	Chaffinch 1	11	15/5/2006
		216[d]	22	22	98	116	101	16	Chaffinch 1	11	15/5/2006
		216[d]	22	22	98	116	101	16	Chaffinch 1	11	15/5/2006
353	ST5	7	2	5	2	10	3	6	Starling 1	4	19/10/2006
		7	2	5	2	10	3	6	Starling 1	4	19/10/2006
177	ST177	17	2	8	5	8	2	4	Starling 1	4	19/10/2006

[a] Allele numbers were assigned by comparing sequences with sequences in the public MLST profile database (http://pubmlst.org/campylobacter). STs and clonal complexes were also assigned by comparison with STs and clonal complexes in the MLST database. Novel sequences and STs were submitted to the database, and new allele or ST numbers were assigned.

[b] U, not assigned to a clonal complex.

[c] Novel ST in this study.

[d] Novel allele in this study.

[e] The number indicates the individual from which the isolate was derived.

[f] Location at which the bird was sampled or found dead. Locations 1 to 4 and 6 to 11, mixed pasture and arable farmland on the Cheshire plain in northwest England; location 5, a small town in Cheshire in northwest England; location 12, salt marsh habitat in the Mersey estuary in northwest England; locations 13 and 15, Morecambe Bay estuary in northwest England; location 14, Dee estuary in northwest England.

Six of the 15 STs identified were also novel (ST3001, ST3002, ST3003, ST3274, ST3275, and ST3276) (Table 2). The most common ST and the most common clonal complex were ST45 and the ST45 complex, which were identified for 19% of the isolates from three wild bird individuals (a moorhen, a barn owl, and a magpie) from three separate locations (Table 2). Twenty-five isolates (69%) were assigned to a previously described clonal complex when their allelic profiles were compared with those in the larger public MLST database for isolates, and 11 isolates (31%) could not be assigned to a clonal complex (Table 2). Four of the six novel allele profiles identified were among the profiles that could not be assigned to a clonal complex. The number of STs in each clonal complex ranged from one (ST42, ST45, ST177, ST353, and ST1275

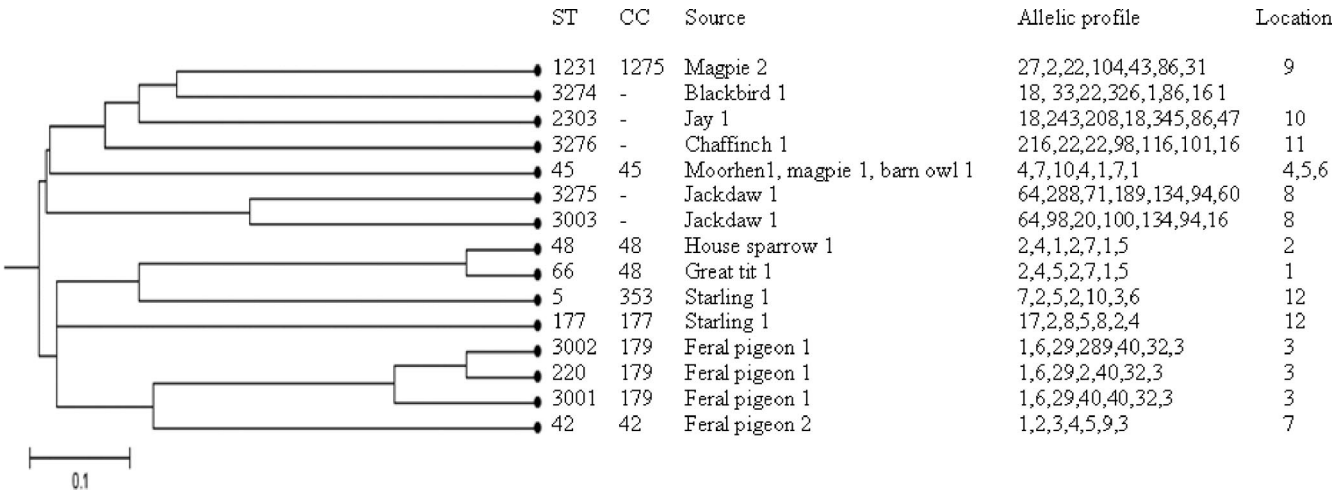

FIG. 1. UPGMA dendrogram based on a distance matrix of pairwise differences in MLST allelic profiles of *C. jejuni* isolates used in this study. Allele numbers were assigned by comparing sequences with sequences in the public MLST profile database (http://pubmlst.org/campylobacter). Locations 1 to 4 and 6 to 11 were mixed pasture and arable farmland on the Cheshire plain in northwest England. Location 5 was a small town in Cheshire in northwest England. Location 12 was a salt marsh habitat in the Mersey estuary in northwest England. Locations 13 and 15 were in the Morecambe Bay estuary in northwest England. Location 14 was in the Dee estuary in northwest England. CC, clonal complex.

complexes) to three (ST179 complex) (Table 2). The ST of the clonal complex founder strain was the most predominant ST in four of the clonal complexes (ST42, ST45, ST48, and ST177 complexes). However, in three of the clonal complexes, the founder ST was not present in the isolates (ST179, ST353, and ST1275 complexes) (Table 2). For the majority (77%) of the wild bird individuals sampled, all of the isolates recovered had the same ST. However, for 23% of the individuals sampled, isolates with more than one ST were recovered from the same sample.

Relatedness and population structure of *C. jejuni* isolates. Using the criterion that members of a group should share five loci with at least one other member of the group, analysis of the isolates collected during this study using the burst algorithm grouped eight isolates with five STs into two groups. Group 1 contained all isolates of ST48 and ST66, and group 2 contained isolates of ST220, ST3001, and ST3002. The founder strains could not be predicted for either of these groups due to the small number of STs for each group. The grouping of STs as determined by burst analysis was confirmed by construction of a UPGMA dendrogram based on allele profiles (Fig. 1). Isolates in group 1 (ST48 and ST66) differed from each other by one allele (*gltA*). ST66 was represented by one isolate from a great tit sampled on a dairy farm in Cheshire (location 1) (Table 2). ST48 was represented by four isolates from an individual house sparrow sampled on a beef farm in Cheshire (location 2) (Table 2). These two farms were separated by 5 km, and the samples were collected 6 months apart. Isolates in group 2 differed from each other by one allele (*glyA*). The three isolates in this group, one each of ST220, ST3001, and ST3002, were isolated from the same feral pigeon that was sampled at location 3. This bird was the only *C. jejuni*-positive individual sampled at this location. The remainder of the STs identified in this study were determined by burst analysis to be singletons. Using less stringent criteria, the UPGMA dendrogram grouped isolates with ST3275 and ST3003 together, which differ from each other by three alleles. Isolates with these STs were recovered from one jackdaw. Two isolates that had only one allele in common (ST5 and ST177 isolates) were detected in a sample collected from one starling.

The I^S_A for the 15 STs detected during this study was 0.366. The observed variance was greater than the maximum variance obtained in 1,000 trials ($P = 0.000$), indicating that there was significant linkage disequilibrium. This suggests there was a degree of clonality in the study population of *C. jejuni* isolates. The dominant clonal complex in this study was the ST45 complex. The I^S_A for all STs in the ST45 clonal complex in the public MLST database (373 isolates with 180 different STs) was 0.003, indicating that there was no significant linkage disequilibrium, which may suggest that recombination within this clonal complex is common.

Comparison with a wider population of *C. jejuni* isolates. *C. jejuni* isolates from our study were compared with all *C. jejuni* strains isolated from wild bird sources for which there are data in the public MLST database (last database query, 14 August 2008). The larger data set included 234 isolates with 177 different STs isolated from wild birds in Europe, North America, and Australasia between 1982 and 2007. Thirteen of the 15 STs identified during our study had not been reported for wild birds in the public MLST database previously. Only isolates of ST42 and ST45 recovered from wild birds were in both our data set and the larger data set. The I^S_A for STs belonging to the larger data set was 0.314, indicating that there was significant linkage disequilibrium in the group of STs reported for wild bird sources in the public MLST database.

Six (40%) of the STs identified in our study have previously been associated with either sporadic cases or outbreaks of human gastroenteritis (ST5, ST42, ST45, ST48, ST66, and ST2303). Isolates of ST66 and ST2303 in the public MLST database have been reported solely from human stool samples from patients with sporadic cases of gastroenteritis. In addition to isolation from human gastroenteritis cases, isolates of ST42, ST45, and ST48 have been recovered from ruminant meat, offal, animal carriers, and environmental samples. Isolates with

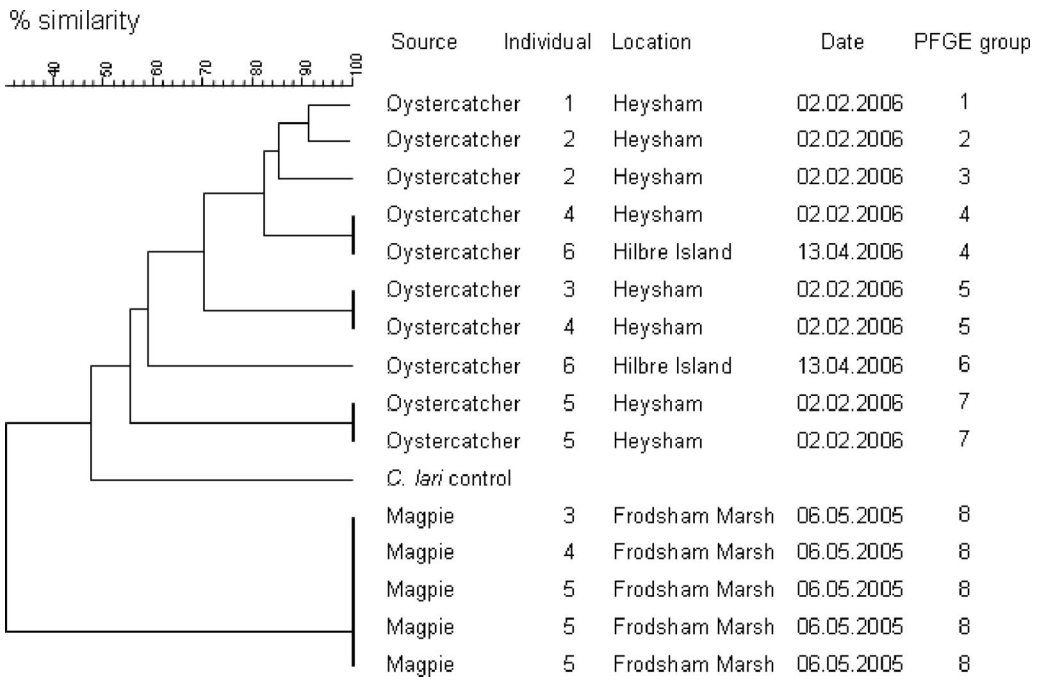

FIG. 2. Dendrogram showing the genetic similarity between *C. lari* isolates digested with the SmaI restriction enzyme. The level of similarity between PFGE banding patterns was computed using the Dice similarity coefficient method with a 2% tolerance window.

ST45 and ST48 have also been isolated from poultry meat and carriers. One isolate with ST5 has been recovered from a pig carrier, as well as from human gastroenteritis cases. Isolates with ST177, ST220, and ST1231 in the public MLST database have been recovered from environmental samples, including samples of environmental water and samples of sand from bathing beaches. However, isolates with other STs in the ST177, ST179, and ST1275 complexes, to which STs identified in this study belong, have previously been recovered from both wild bird sources and stool samples from humans with sporadic cases of gastroenteritis.

PFGE. PFGE was carried out for 15 of 19 PCR-positive *C. lari* isolates. Four of the PCR-positive isolates could not be revived from storage at −80°C. Isolates that were subjected to PFGE were from nine wild bird individuals belonging to two species, magpies and oystercatchers. The magpie individuals were sampled on the same date in a salt marsh habitat. The samples from oystercatcher individuals were collected from two oystercatcher flocks in two estuaries separated by approximately 95 km on two dates 2 months apart. PFGE revealed eight SmaI banding patterns that were labeled PFGE groups 1 to 8 (Fig. 2). These SmaI PFGE groups were confirmed by PFGE after digestion with the KpnI restriction enzyme. The level of genetic similarity within each PFGE group was very high (>95%). One PFGE pattern was identified for *C. lari* isolates from all magpie individuals sampled in the same area on the same day (PFGE group 8); these isolates, including multiple isolates from one individual, showed 100% genetic similarity to each other. Seven PFGE patterns were identified for 10 isolates collected from six oystercatcher individuals (PFGE groups 1 to 7), and the overall genetic similarity between the groups was 56%. Multiple *C. lari* isolates were isolated from fecal samples from four oystercatcher individuals. Of these four individuals, only one oystercatcher yielded multiple isolates with the same PFGE banding pattern (PFGE group 7). Analysis of the only *C. lari*-positive oystercatcher from a population sampled in the Dee estuary during April 2006 (oystercatcher 6) (Fig. 2) yielded two isolates with different PFGE patterns (PFGE groups 4 and 6); one of these isolates had a banding pattern identical to that of an isolate obtained from a different oystercatcher (oystercatcher 4) sampled during February 2006 from a different population 95 km north of the Dee estuary (PFGE group 4). PFGE group 5 contained two isolates from different individuals in the same population that had identical PFGE banding patterns. Conversely, PFGE groups 2 and 3 each consist of one isolate showing 85% genetic similarity, and both isolates were isolated from a sample from one individual oystercatcher. For all *C. lari* isolates, the PFGE banding patterns suggested that the overall level of genetic similarity was 30%. All *C. lari* isolates were found to be urease negative.

DISCUSSION

The overall prevalence of *Campylobacter* spp. for all wild birds sampled in this study was 1.4% (95% CI, 1.0 to 2.0%). However, the prevalences of different *Campylobacter* spp. in members of different wild bird taxonomic families were heterogeneous, ranging from 0% to 33%. Compared with the results of other studies describing *Campylobacter* in wild bird populations, which reported prevalences ranging from 2% to 50% (5, 21, 25, 29, 34, 47, 48), the prevalence reported in this study is relatively low. Prevalence estimates are likely to vary between studies due to the use of different sampling regimens and culture methods, which vary in sensitivity. Furthermore, there is evidence that the survival of *Campylobacter* spp. in fecal

samples from different wild bird species is variable (48). The extent to which survival of *Campylobacter* spp. varies in fecal samples from most wild bird species is not known. Previous studies of *Campylobacter* occurrence in wild bird populations have reported differences in prevalence between different wild bird life stages, including breeding, migration, molting, and wintering, and differences depending on the feeding guild to which wild bird species belonged (21, 25, 29, 47, 48). It is clear that the ecology of *Campylobacter* infection in wild birds is complex, involving many intrinsic and extrinsic factors, and unless such factors are carefully considered, comparisons of prevalence values between studies may be misleading.

High prevalences of *Campylobacter* spp. in wild birds have often been interpreted as evidence that there is nonpathogenic coexistence of *Campylobacter* spp. and wild bird hosts (30, 31). The findings of this and previous studies suggest that this may be the case for some, but not all, wild bird taxa. In this and similar studies, *Campylobacter* spp. were not found to be equally distributed among all wild bird species investigated (21, 25, 34, 47). This might indicate that certain species of *Campylobacter* have coevolved with specific wild bird taxa. The occurrence of *Campylobacter* in wild birds is rarely associated with disease and was never associated with obvious disease in our study. Considering that campylobacters are known to survive in the environment (1, 3, 26, 32), it is more likely that wild bird taxa which share particular ecological factors (for example, habitat, diet, and/or behavioral characteristics) are exposed to *Campylobacter* more frequently than other wild bird taxa.

Despite the fact that there is no apparent association between specific *C. jejuni* strains and wild birds, there are clonal complexes that appear to be associated with environmental samples, which have also been detected in wild birds (6, 10, 30). Five of the 15 STs identified in this study (ST177, ST220, ST1231, ST3001, and ST3002) belonged to clonal complexes associated with environmental samples in previous studies (ST177 complex, ST179 complex, and ST1275 complex). These STs may be better adapted to survive in the environment, or the results may simply reflect contamination of the environment by bird feces. Until recently, it was thought that such strains may be nonpathogenic in humans, as they had never been isolated from human campylobacteriosis cases. However, recently, strains belonging to these clonal complexes have been isolated from sporadic cases of human gastroenteritis (30, 42).

Forty percent of the STs detected were unique to this study. A previous study investigating the occurrence of *C. jejuni* in wildlife and wild birds in a farmland habitat also found a high proportion of unique STs (15). None of the STs unique to this study have been reported for domestic animals or humans (last public MLST database query, 14 August 2008). However, livestock-associated strains were detected in wild bird samples, probably as a result of wild birds sharing a common environment or interacting with livestock. Therefore, if wild birds act as a significant source of *Campylobacter* strains for livestock, as has been suggested previously (4, 7), it might be expected that the novel strains detected in wild birds would also have been detected in livestock. The apparent absence of these novel strains in livestock may suggest that the predominant direction of infection is from livestock to wild birds.

Two of the novel strains, strains with ST3001 and ST3002, differed from each other and previously recognized ST220 strains by one locus (*glyA*). Strains with all three STs were isolated from one pigeon. These unique STs may represent recent variants of the previously described ST220, which may have arisen due to in vivo genetic recombination between strains in the host. A further two novel strains were isolated from one jackdaw (ST3003 and ST3275), and they shared three of seven alleles. It is possible that these strains are variants of a common strain, that one strain evolved from the other, or that the jackdaw acquired the two strains from one or multiple sources. In addition to coinfection with genetically similar strains, strains with two previously described unrelated STs (ST177 and ST5) were isolated from the fecal sample from one starling. It is thought that coinfection with more than one strain of *C. jejuni* may lead to recombination between strains and evolution of new strains within hosts (9, 38), which may explain the occurrence of unique genetically diverse STs in the wild bird populations examined.

It is possible that the unique STs identified in this study are parts of larger clonal complexes that are currently unidentified due to the small number of studies that have characterized wild bird isolates using MLST. Analysis of all *C. jejuni* isolates from wild birds in the public MLST database ($n = 234$; last database query, 14 August 2008) indicated that there is a degree of clonality in this *C. jejuni* population (I^S_A, 0.314) and that some STs are members of small complexes (STs having five or more MLST loci in common). However, the majority of STs were singletons or related to one other strain, suggesting that there is a high degree of genetic diversity among isolates from wild birds. Furthermore, 13 of the 15 STs identified in our study have not been reported previously for wild birds. This suggests that numerous strains of *C. jejuni*, many of which have not been identified yet, are carried by wild birds. Further studies would be useful for determining if the singleton STs are indeed singletons or parts of larger clonal complexes.

C. lari was detected in 13 of 2,084 (0.6%; 95% CI, 0.4 to 1.1%) wild birds sampled, and the occurrence in different wild bird taxa was not very heterogeneous since this species was obtained from only 6 of 99 wild bird species that were sampled. Accordingly, due to the small number of isolates examined, the results presented here should be treated as a starting point for further examination rather than a final conclusion. The prevalence of *C. lari* was greatest among wading birds (Haematopodidae, 7.8%; Scolopacidae and Charadriidae, 1.3%), followed by gulls (Laridae, 4.4%) and corvids (Corvidae, 3.0%). Very low prevalences were detected for ducks, geese, and swans (Anatidae, 0.4%) and finches (Fringillidae, 0.4%). These findings are largely in keeping with the findings of previous studies (47, 48). *C. lari* has long been associated with members of the gull family (16, 23, 24, 41) and was isolated from gulls in this study, but it was isolated less frequently from gulls than from wading birds. However, more wading birds than gulls were sampled in our study; consequently these observations should be treated as a starting point for further examination.

The majority of *C. lari* isolates were obtained from estuarine or estuarine marsh habitats. In these habitats, *C. lari* was most commonly isolated from Eurasian oystercatchers (*H. ostralegus*) and magpies (*P. pica*). Eurasian oystercatchers are gregarious birds that feed predominantly in estuaries on bivalve mollusks, which they locate by probing in estuarine mud with

their bills. Studies investigating the occurrence of *Campylobacter* spp. in shellfish detected a high incidence of genetically diverse *C. lari* in mussels from The Netherlands, Germany, Denmark, England, and Ireland (12, 51). It is possible that there is a cycle between oystercatchers and their bivalve prey whereby oystercatchers acquire *C. lari* from eating bivalve prey and subsequently shed *C. lari* into the estuarine environment in which mollusks live.

PFGE analysis of the *C. lari* isolates from oystercatchers in this study revealed that these isolates are genetically diverse. Multiple isolates from the same fecal sample with different PFGE banding patterns were detected for three birds, showing that individual birds can harbor more than one strain of *C. lari* and illustrating the high level of genetic diversity even in this small number of isolates. A high prevalence of genetically hypervariable urease-positive thermophilic strains of *C. lari* was recently reported in a population of Swedish shore birds (48). The isolates examined in our study were urease negative; nevertheless, they showed the same high levels of genetic diversity. It has been suggested that generation of genetic diversity is likely an adaptive strategy employed by campylobacters to increase their chances of surviving environmental stresses that are likely to be encountered during host-to-host transmission (38). However, *C. lari* isolates with the same PFGE banding pattern were isolated from fecal samples from three oystercatcher individuals. Similarly, *C. lari* isolates having the same PFGE pattern were isolated from three magpies sampled in the same salt marsh habitat. This may indicate that despite high levels of genetic diversity among *C. lari* isolates, there are some strains that are more environmentally persistent than other strains and consequently more widely distributed in the environment. Alternatively, the birds from which these isolates were derived may have acquired them from the same source. However, due to the small number of isolates examined it is not possible to draw solid conclusions from these data; rather, hypotheses for further investigation are created.

In conclusion, MLST analysis of *C. jejuni* isolates revealed that wild birds carry both livestock-associated and unique strains of *C. jejuni*. The occurrence of livestock-associated strains in wild birds may suggest that these birds can act as a source of *C. jejuni* for livestock. However, the apparent absence of unique wild bird strains of *C. jejuni* in livestock may suggest that the direction of infection is predominantly from livestock to wild birds and less often from wild birds to livestock. Further studies of the *C. jejuni* occurrence in wild bird populations would be valuable for determining if unique strains identified in this and other studies belong to larger clonal complexes.

PFGE analysis revealed both genetically diverse and clonal populations of *C. lari* in two different wild bird species sharing the same habitat. The differences are likely to be influenced by complex interactions between the ecology of *C. lari* populations and differences in host life history, which warrant further investigation.

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