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***Campylobacter* Excreted into the Environment by Animal Sources: Prevalence, Concentration Shed, and Host Association**

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Abstract

An intensive study of 443 isolates of *Campylobacter jejuni* and *Campylobacter coli* from 2031 fecal samples excreted by animal sources including cattle, sheep, and pigs, a range of wild and domesticated avian species and pets is described. The prevalence found in the majority of animal sources ranged from 22% to 28% with poultry being highest at 41% and cats and dogs lowest (<5%). The average count excreted for each animal source was found not to be significantly different ranging from approximately 10² to 10⁵ cfu/g. Multilocus sequence typing (MLST) identified phylogenies that exhibited host specificity. A number of clonal complexes (CCs) and sequence types (STs) were characteristic of particular hosts (e.g., CC-179, ST-637, and ST-1341 found only in pigeons and gulls). Analysis of genetic distance demonstrated numerous significant differences in the distribution of MLST types (CC, ST, and allele) between animal sources. Host association was quantified using structure that correctly assigned the nine animal sources with accuracies of 28%, 24%, and 55% at the CC, ST, and allele levels, respectively. This is substantially higher than would be expected by random allocation (11%) but farmyard poultry had the lowest assignment accuracy (13%, 13%, and 21%) suggesting that isolates were shared with a wide range of other animals. This study demonstrates the link between MLST type and host and provides data that can be used in risk assessment and food attribution models. Further, it demonstrates the applicability of MLST to characterize *Campylobacter* strains from a broad range of environmental sources.

Introduction

Human Campylobacteriosis is a zoonosis and the leading cause of recognized bacterial gastroenteritis in industrialized countries (Olson *et al.*, 2008). The majority of human infections (>80%) are due to *Campylobacter jejuni* with the remainder being predominantly *Campylobacter coli*. Recent reported incidence rates were 95.3 per 100,000 in Scotland in 2006 (Locking *et al.*, 2007) and 12.7 per 100,000 in the United States in 2007 (Centers for Disease Control and Prevention, 2007). These rates are likely to be the substantial underestimates of the actual disease burden because it is estimated that only 1 in 7 cases are

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reported in the United Kingdom (Wheeler *et al.*, 1999) and only 1 in 38 cases in the United States (Mead *et al.*, 1999).

Campylobacter are found in the intestines of many wild, domestic, and farm animals (Moore *et al.*, 2005). Cattle, sheep (Stanley and Jones, 2003), poultry (Bull *et al.*, 2006), and pigs (Boes *et al.*, 2005) are reservoirs, and they harbor high levels of strain diversity (Colles *et al.*, 2003). Companion animals, including cats and dogs, also carry *Campylobacter* (Lee *et al.*, 2004; Workman *et al.*, 2005). *Campylobacter* excreted from all reservoirs can be found throughout the environment including soil (Santamaria and Toranzos, 2003), beach sand (Bolton *et al.*, 1999), sewage (Jones, 2001), and groundwater (Stanley *et al.*, 1998).

Foodborne pathways of human *Campylobacter* infection have been intensively studied, with retail chicken being the most frequently implicated factor. As many as 90% of the birds (Gormley *et al.*, 2008) and 87% of the breast fillets (Luber and Bartelt, 2007) are contaminated according to the retail chicken surveys in Scotland and Germany, respectively. Chicken consumption, especially when undercooked or cross contamination with other foods (Rodrigues *et al.*, 2001), is a key risk factor for infection in several case-control studies and also the 1999 dioxin crisis in Belgium where chicken was removed from sale because the dioxin-contaminated feed coincided with a 40% reduction of human *Campylobacter* cases (Vellinga and Van Looock, 2002). Other vehicles of transmission include raw milk (Eberhart-Phillips *et al.*, 1997; Michaud *et al.*, 2004), salad vegetables (Evans *et al.*, 2003), barbequed meat (Kapperud *et al.*, 2003; Neimann *et al.*, 2003), and untreated drinking water (Kapperud *et al.*, 2003).

In contrast, environmental pathways of human *Campylobacter* infection remain poorly understood with comparatively few risk factors identified. Those included are the ingestion of contaminated water during swimming (Schonberg-Norio *et al.*, 2004), visiting or living on a farm (Friedman *et al.*, 2004), and of children living in the countryside (Ethelberg *et al.*, 2005). The multiplicity of potential environmental sources, the rarity of environment-sourced outbreaks to help infection source identification (Cowden, 1992; Miller and Mandrell, 2005), the limited spatial and temporal scales of most reported studies, and the use of different typing methods yielding strain categories that are difficult to compare (Frost *et al.*, 2002) all conspire to hinder further elucidation of environmental sources of human *Campylobacter* infections.

One approach that promises further insights into environmental sources of *Campylobacter* infection is to identify strains characteristic of particular hosts and estimate the fecal shedding from those hosts into the environment. This approach is most effective when strains are categorized using a universal typing method based on portable data (i.e., readily allowing results from different labs to be compared and also storage on an electronic database). For many bacterial pathogens, including *Campylobacter*, these requirements are satisfied by multilocus sequence typing (MLST) (Dingle *et al.*, 2001a, 2001b). *Campylobacter* strains characterized using MLST are categorized as sequence types (STs), and STs and their component alleles are compared using an open-access database (<http://pubmlst.org/campylobacter/>). Host specificity of *Campylobacter* STs is evident in isolates from farm animals and wildlife (French *et al.*, 2005) and from cattle, sheep, and poultry (McCarthy *et al.*, 2007). However, these studies were of limited spatial and temporal scales, whereas human *Campylobacter* infection is usually reported at the national level.

The aim of this study was to characterize farm animals, pets, and wild birds from a national-scale collection in Scotland according to the prevalence and concentration of *Campylobacter* in their feces and to the extent of association of the different hosts by categorizing *Campylobacter* strains using MLST.

Materials and Methods

Fecal samples from potential *Campylobacter* hosts were collected in rural and urban areas during August 2005 to September 2006. The rural areas included 116 randomly selected farms (sampled once each) in NE and SW Scotland. The farms were chosen as follows. The postcode districts (indicated by the first four digits of the postcode) in the rural areas were tabulated, the central town or village was identified, farmers listed in the local telephone directory (www.yell.com) under that location were contacted by phone, and one willing farmer in each postcode district randomly chosen for each collection visit. NE and SW areas were visited concurrently. The species sampled in rural areas were cattle, sheep, pigs, dogs, farmyard poultry (hens), pigeons, corvids, and gulls. The urban areas were sites in the cities of Edinburgh (10 sites), Glasgow (8), and Aberdeen (17) with high risks of *Campylobacter* transmission to people, for example, parks, waterfronts, open-air eating areas, public squares, and small petting farms. The species sampled in the urban areas were dogs, cats, feral pigeons, gulls, ducks, geese, and swans. Avian samples from unknown sources were categorized as birds. In all cases, fresh fecal mammalian (25 g) and fresh avian (<5 g) grab samples were collected ($n = 2023$). The grab samples involved placing a sterile plastic bag inside-out over the hand and then “grabbing” the sample. Some smaller avian samples were collected using a sterile swab moistened with saline. Samples were transported chilled (4°C) to the laboratory for immediate analysis.

Identification and enumeration of *Campylobacter*

Fecal aliquots (10 g) were homogenized (10:90 w:v) in *Campylobacter* enrichment broth (see below) and 0.1 mL decimal dilutions plated directly onto the modified *Campylobacter* Blood-Free Selective Agar Base (CCDA base, CM0739; Oxoid, Basingstoke, UK) with CCDA selective supplement (SR 155; Oxoid) for target enumeration (minimum count was therefore 100 cfu/g). The swabs were individually tested by adding a portion of a measured 10 mL volume enrichment broth into the swab tube. The sample was gently vortexed, and the contents of the swab tube and the swab were placed into the remaining enrichment broth and gently mixed. Two further dilutions were prepared from this sample and then 0.1 mL plated for enumeration. The remainder of the sample was incubated for presence or absence. The presence or absence of *Campylobacter* was ascertained by incubating the remaining enrichment broth microaerobically in an atmosphere of 10% CO₂, 5% O₂, balance N₂, 100 mL volumes of nutrient broth base (Mast, Bootle, UK) with 5% horse blood, growth supplement (Mast Selectavial SV61), amphotericin (2 µg/mL), cefoperazone (15 µg/mL), and trimethoprim (10 µg/mL); polymixin B (2500 IU/L) and rifampicin (5 µg/mL) were added, and the broths incubated for 2 days at 37°C (Bull *et al.*, 2006). All antimicrobials were purchased from Sigma-Aldrich (Gillingham, UK). Enrichment broths (0.1 mL) were plated onto mCCDA agar incubated microaerobically for 2 days at 37°C. Five colonies were taken from each plate and presumptively identified as *Campylobacter* microscopically (Gram stain) and by agglutination with Microscreen latex (Microgen, Camberley, UK). Individual colonies were stored (−80°C, nutrient broth, glycerol added to 15% [v/v]) for MLST. *Campylobacter* presence was confirmed using isolate ST as determined by MLST.

Genotyping

Archived isolates were plated from frozen onto CCD agar and incubated microaerobically for 48 h at 37°C. Bacterial DNA was prepared and MLST performed as previously described (Gormley *et al.*, 2008). Sequences were assembled using STARS software available at <http://pubmlst.org> and novel alleles, and STs were submitted to the *Campylobacter* MLST database at this website.

Analysis of prevalence

Campylobacter prevalence values with 95% binomial confidence intervals (CI) were calculated using Microsoft Excel. Differences in prevalence values between sources were tested for statistical significance using Fisher's exact test (www.quantitativeskills.com/sisa/statistics/twooby2.htm), and odds ratios (OR), CI, and *p*-values obtained. OR (Bland and Altman, 2000) were defined as the odds of a *Campylobacter* being found in a particular source divided by the odds of being found in a different source. An OR value >1 indicates a higher prevalence in the first source.

Analysis of microbiological enumeration

Counts were estimated for each source and the average calculated. The 95% CI were determined using a bootstrap method that involved sampling the original data with replacement. This process was repeated 10,000 times using Poptools (www.cse.csiro.au/poptools/). Enumerations were compared between all pairs of sources. Briefly, the difference of the average counts for each pair of hosts was calculated and tested for significance in Visual Basic Application under Excel (VBAE) by comparing this difference with a distribution of 10,000 differences obtained by randomizing the data without replacement (Manly, 2007).

Clonal frame

The clonal genealogy of STs was estimated using the model-based approach in determining bacterial microevolution: clonalframe (Didelot and Falush, 2007). This model calculates clonal relationships with improved accuracy distinguishing point mutations from imported chromosomal recombination events, the source of the majority of allelic polymorphisms. Analysis was carried out on concatenated sequences representing 150 STs, from 16 animal species. The program was run with 50,000 burnin followed by 50,000 subsequent iterations. The consensus trees represent combined data from three independent runs with 75% consensus required for inference of relatedness.

Analysis of diversity

Rarefaction analysis (Heck *et al.*, 1975) was used to estimate the diversity of each of the nine animal sources (rural and urban combined) that had 10 or more MLST-typed isolates. The method was implemented in VBAE and incorporated MLST data at the levels of clonal complex (CC—a group of STs whose members are linked to at least one other member by being identical for six of the seven MLST genes) and ST. Diversity of strains between any two sources was carried out as follows. Rarefaction estimated the number of new types found in a particular number of isolates for each of the individual sources and the difference in diversity was determined by subtraction. Testing for significance was performed using a randomization test (Manly, 2007) with correction for differences in sample size. Briefly, the data from the two sources were randomized without replacement, the rarefaction was then rerun and the difference in diversity calculated. This was repeated 10,000 times. The posterior distribution of diversity differences obtained was then compared with the diversity difference calculated between the two original animal sources to obtain the level of significance (*p*-value).

Analysis of genetic distance between populations

Pairwise comparisons of the genetic distance between each of the nine animal sources (rural and urban combined) were calculated using the genetic distance, d_1 , described by Manly (1985).

$$d_1 = 0.5 \sum_i |p_i - q_i|$$

where p_i and q_i are the proportions of a particular genotype found in each of the animal sources under comparison. When $d_1 = 1$, there are no genotypes in common and when $d_1 = 0$ the two animal sources have the same distribution of genotypes. Genetic distance was determined at the level of ST, CC, and allele level (for the allele level the right-hand side of the equation was modified to sum the contribution across all seven MLST alleles, which was then divided by seven). The statistical significance of the genetic distance was found using the randomization test method as described above. Analysis of molecular variance was also performed and is supplied in the Supplemental Material.

Analysis of host association

Structure software (Pritchard *et al.*, 2000) was used to assign MLST isolates to animal source populations (rural and urban combined) based on the difference in frequency of genotypes between populations. The methodology used was a modification of that described previously (McCarthy *et al.*, 2007). Briefly, a nonadmixture model (i.e., isolates come from a single-source population) with uncorrelated gene frequencies between populations was used. A jackknife method (15,000 iterations of the program, implemented in C++) was used in assigning unknown MLST isolates to animal sources, and the probabilities of assignment were determined. Then, 10,000 Monte Carlo steps were used to calculate the average proportion, and corresponding 95% CI, of the correct assignment to the host animal sources.

All p -values obtained by statistical techniques were corrected by sequential Bonferroni correction where multiple comparisons were carried out. This involved multiplying the p -value obtained by the number of comparisons with the resulting p -value being considered statistically significant when $p < 0.05$. The VBAE programs are available by request.

Results

Prevalence and microbiological enumeration

Campylobacter prevalence was lowest in dogs and highest in poultry (Table 1). The main farm animal sources (cattle, sheep, and pigs) had similar prevalence values (Table 1) and had OR not significantly different from 1.0 ($p > 0.05$), whereas dogs had a lower prevalence than most other hosts ($p < 0.05$, Tables 1 and 2). *Campylobacter* concentrations were lowest in poultry and highest in ducks, sheep, pigeons, and geese. Sheep had concentrations approximately 10-fold higher than cattle (Table 1). None of these differences was significant in pairwise comparisons ($p > 0.05$, data not presented).

Clonal frame

Clonal frame readily distinguished *C. jejuni* from *C. coli* STs (Fig. 1A). The *C. coli* tree consisted of one major and three minor clades (Fig. 1B, for ease of viewing the STs from all avian species except poultry are represented by black circles). All minor clades comprised exclusively avian species excluding poultry. The main clade had pig and poultry STs widely distributed and also contained cattle, avian, and sheep STs. Hence, the *C. coli* tree provides evidence of genetic segregation/host specificity between animal sources. The *C. jejuni* tree (Fig. 1C) comprised one major clade and one minor (with cattle, sheep, and poultry STs) clade. The major clade had a broad distribution of avian species excluding poultry STs. The sheep and poultry STs were widely distributed but where avian (excluding poultry) STs are

common other animal sources are rare, and *vice-versa*. This further demonstrates genetic differences between animal sources.

Strain diversity

None of the ST rarefaction curves reach a plateau indicating that the ST diversity detected in each host within this study is a proportion of the total diversity that that particular host excretes into the environment (Fig. 2B). In contrast, the CC rarefaction curves (Fig. 2B) for cattle and sheep show more evidence of a plateau indicating that a high proportion of the total CC diversity from these hosts was sampled. The rare-faction suggests that pigeons have the lowest MLST diversity, while a number of hosts (e.g., other birds) appear to have high diversity. Pairwise comparisons of diversity between hosts (Table 3) show that sheep, pigs, and pigeons have a lower diversity than unidentified birds ($p < 0.05$). At the ST level, only pigeons have a lower diversity than birds and geese but here poultry shows a greater diversity than pigeons ($p < 0.05$). The relatively high diversity in birds at both CC and ST levels is not surprising since birds will comprise a number of avian species that are likely to have a number of host-associated MLST types.

Genetic distance

Table 4 presents standardized pairwise genetic distances between animal sources at the levels of CC, ST, and allele. The greatest number of statistically significant pairwise differences was found at the allele level (28/36) compared with 24 at the level of CC and 19 by ST. Ducks and poultry generate the lowest number of significant pairwise differences (8 and 10) when comparing all three levels of the analysis. Pigeons were found to be significantly different from the other animal sources at all three levels with CC-179 dominating (Fig. 3) and being only found occasionally elsewhere (in gulls). Results from analysis of molecular variance show similar pairwise differences (see Supplemental Material as was obtained using the standardized genetic distance.

Host association

Figure 3 illustrates the distribution of types by host at the levels of CC and ST. Table 5 shows the host assignment accuracy from structure. Overall, each of the three levels (CC, ST, and allele) of analysis demonstrated better classification accuracy than would be expected at random (11.1%) with allele level analysis performing best (54.7%). Poultry exhibited the lowest classification accuracy (12.5–20.9%), while pigeons showed the greatest host association (60.1–77.5%). Farm animal sources (cattle and pigs) gave average (18.4–30.0%) classification by CC and ST but exhibited substantially higher host association at the allele level. Sheep gave similar CC and ST values as cattle and pigs, but the allele level showed only a moderate increase (40.2%).

Discussion

Structure showed that host association was strongest at the allele level, in agreement with previous work (McCarthy *et al.*, 2007). Here, however, an accuracy of 55% is obtained for identifying nine animal sources, whereas in the McCarthy study a value of 58% was reported for three sources (cattle, sheep, and pigs). The isolates in the current study were obtained over a discrete time interval (15 months) and within a restricted geographical area (specific areas within Scotland) that may partly explain the relatively high accuracy for the large number of animal sources. In addition, the wider range of animal sources studied here appear to have a number of genotypes that show host restriction or appear far more frequently in one source than in others (e.g., in gulls and pigeons; Fig. 3). The reasons for this may be attributed to mechanisms of isolation, host adaptation, or genetic drift. Further, an additional analysis was carried out (data not presented) applying structure solely to cattle

and sheep isolates and a host association of 65% was obtained. This is closer to that obtained in previous studies (McCarthy *et al.*, 2007). This suggests that the *Campylobacter* types found within cattle and sheep are more similar to each other than, for example, between bovids and wild birds.

At all three levels of analysis (CC, ST, and allele) poultry isolates exhibit the poorest degree of host association of all animal sources tested. However, the number of poultry isolates is small ($n = 24$), but the results do indicate that poultry may have the ability to acquire *Campylobacter* from a number of different animal sources. Pigs exhibited a very high degree of host association at the allele level (95%) compared with the ST (18%) and CC (27%) levels. Pig isolates were predominantly *C. coli* (CC-828) but exhibited a number of alleles on the *tkt* locus (data not presented) that are either absent or rare in *C. coli* isolates from other animal sources (e.g., sheep and cattle). These pig-associated alleles include *tkt*-35 and *tkt*-44 that have previously been found to be associated with pigs (Miller *et al.*, 2006).

The lineages identified here for *C. jejuni* and *C. coli* are in agreement with previous work (Sheppard *et al.*, 2008) that also demonstrated that *C. coli* has one major and a number of minor clades. Within both species, host association is apparent through the clustering of STs and this is particularly apparent for the avian (excluding poultry) hosts, originally reported by French (2007).

The rarefaction analysis indicates that only a relatively small proportion of full genetic diversity has been tested. This is particularly the case at the ST level. This gives an indication of the sampling intensity required to instigate a surveillance program to monitor the *Campylobacter* population in animal hosts which is essential to understand whether *Campylobacter* types remain relatively stable within a population or are subject to change over time as can readily be observed with both *Salmonella* (Cogan and Humphrey, 2003) and *Campylobacter* (Gormley *et al.*, 2008) in poultry. Further, a comprehensive survey has the potential to elucidate how strains may be circulating and evolving in animal populations and which subsequently cause disease in humans by either foodborne or environmental routes.

The CC most commonly found in gulls (CC-1275) has also been reported in gulls from Sweden and Australia (PubMLST database). A number of the STs from these international gull isolates were found in the current study but the most common, ST-637 in the CC-1275 complex here, has only been reported previously from a chicken in Denmark. It is also worth noting that in the current study this ST was found in all three cities demonstrating its widespread nature in Scotland. The pigeon-associated CC (CC-179) has previously been reported (PubMLST database) in sand from UK bathing beaches and from wild birds of UK origin. The two most common STs (ST-220 and ST-2209) have been found in bathing beach sand and wild birds (including a pigeon), respectively. In the current study this CC dominated the pigeon isolates from all three cities as well as the rural isolates indicating its ubiquity across Scotland. A previous study (French *et al.*, 2005) of *Campylobacter* in a rural farming environment suggested that ST-61 was overrepresented in cattle compared to birds, rabbit, and water isolates. This agrees with the current study that shows that ST-61 is more common in cattle than pigeons and gulls (see Supplemental Material. The PubMLST database also shows that this ST is well represented in both cattle and sheep but is not present in wild birds. The *C. coli* CC-828 has previously been reported to dominate *Campylobacter* in pigs (Rosef *et al.*, 1983) which is in agreement with results here. It has also been reported (Oporto *et al.*, 2007) that *C. coli* is more common in sheep than cattle, again in agreement with results here. *C. coli* is rare in all the avian species (2/175 isolates) reported here except poultry (7/24 isolates).

Campylobacter MLST types in poultry here are not consistent with those from retail UK chicken from NE Scotland (Gormley *et al.*, 2008). For example, retail chicken data showed two dominant STs (ST-257 and ST-2030) that are absent from the poultry isolates obtained in the current study. This may indicate that strains found in farmyard poultry may not be representative of those in retail poultry where birds are typically farmed in large units, often exceeding 10,000 birds. Further, the source of retail chicken may originate from another part of the United Kingdom. Alternately, the samples tested here may not be representative of the overall population of STs found in poultry, supported by the rarefaction curve (Fig. 3) that does not plateau suggesting that additional sampling would yield further STs.

Both the prevalence and counts of *Campylobacter* in sheep were similar to those reported in previous studies in England (Jones *et al.*, 1999) and Northern Ireland (Madden *et al.*, 2007). However, these values are substantially lower than found in New Zealand (Devane *et al.*, 2005) where prevalences of 76% (sheep), 84–98% (cattle), and 65% (ducks) were reported. These higher prevalence figures may be a factor in the high incidence rates of human campylobacteriosis in New Zealand. Further, we found that Scottish sheep had a number of high shedding ($>10^4$ /g) animals and it has been reported (Matthews *et al.*, 2006) that for organisms such as *E. coli* O157 these “high shedding animals” are considered to be pivotal in maintaining and propagating infection within an animal group. Target enumeration therefore provides useful information on the dynamics of *Campylobacter* in the farm environment. The very low prevalence reported here for *C. coli* and *C. jejuni* in healthy cats (4.5%) and dogs (1.3%) is in agreement with data reported in cats (2%) (Bender *et al.*, 2005) and dogs (0.8%) (Hackett and Lappin, 2003) in the United States and in cats (4%) and dogs (9%) in Australia (Baker *et al.*, 1999).

The number of samples of farmyard poultry is low because free range hens are uncommon in the areas sampled in this study. Currently, the majority of poultry in Scotland (and the United Kingdom) are broilers housed in large sheds. Hence, the amount of fresh fecal material from poultry is not widely distributed ($>95\%$ is incinerated at the end of each growout in Scotland) and as such may play a lesser direct role in transmitting *Campylobacter* in the environment. The prevalence of *Campylobacter* in farmyard poultry (41%) was considerably less than observed in retail poultry (90%) (Gormley *et al.*, 2008) suggesting that poultry in broiler houses have a higher prevalence or that there is substantial cross contamination during processing as previously reported (Allen *et al.*, 2007). The farmyard poultry fecal counts are low compared with broilers (Nauta *et al.*, 2007) and this may be a function of stocking density or perhaps age of the birds. Further, the farmyard poultry fecal counts are of a similar magnitude to that reported on retail poultry meat (majority approximately 10^2 cfu/g; Gormley *et al.*, 2008). However, human infection is more likely through retail poultry as the prevalence is higher and the likelihood of exposure is greater (e.g., through undercooking, cross contamination of other foods in the kitchen as well as direct human contact with chicken meat being more frequent than contact with chicken feces).

The levels of host association reported here together with comprehensive data on prevalence and counts offer the potential of source tracking campylobacter strains through the food chain. This would enable the attribution of human cases to animal hosts and food sources at the population level, currently established for *Salmonella* (Hald *et al.*, 2004). Indeed, a number of papers have recently been published on *Campylobacter* applying source attribution methods (Wilson *et al.*, 2008; Mullner *et al.*, 2009; Sheppard *et al.*, 2009; Strachan *et al.*, 2009). These workers demonstrate that chicken is the main source of infection but that strains from other sources, in particular ruminants but also to a lesser extent wild birds, play a role in infection. Further it has been reported that in rural children there is an excess of cases and that this is due predominantly to nonpoultry sources (French

et al., 2009; Strachan *et al.*, 2009). It should be noted that the etiology of *Campylobacter* is complex and human disease is a consequence of multiple infection pathways including environmental contact (e.g., contaminated drinking water or from farm animals), infection acquired from abroad in addition to food. Consideration should also be given to the point that the infectivity of *Campylobacter* types from different hosts or environments may vary.

There is an ongoing requirement to monitor *Campylobacter* in the environment because the abundance of the major MLST types can change with time (Gormley *et al.*, 2008). It is also likely that the sizes of the reservoirs excreting the organism into the environment will change with time (e.g., UK pig numbers are currently declining) and there is a need for the assessment of agricultural interventions to reduce the burden of *Campylobacter* (e.g., providing advice on dealing with manure), food animals (e.g., improving biosecurity in broiler houses), and food products. We advise that the characterization of *Campylobacter* in the environment as described here should be seen as a preliminary step in understanding the ecology of this organism. The information on the strain types found in different animal hosts together with the prevalence and enumeration data can be used as a basis to develop models that examine the maintenance and cycling of *Campylobacter* in the environment (Skelly and Weinstein, 2003).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 2. Rarefaction curves showing diversity by clonal complex (**A**) and ST (**B**) for animal sources with 10 or more multilocus sequence typing-typed *Campylobacter* isolates.

FIG. 3. Common *Campylobacter* clonal complex (A) and ST (B) types (% of total dataset), each animal source is normalized by its representative number of isolates and the sum of all the grid elements in each figure totals 100% (birds are avian samples of unknown host species).

Table 1
Source, Location, Prevalence, and Concentrations (cfu/g) of *Campylobacter* in Feces from Different Hosts

Host	Prevalence			Campylobacter concentrations (cfu/g)									
	Rural +ve/total	Urban +ve/total	Total prevalence % (95% CI) ^a	0	10 ⁰ -10 ²	10 ² -10 ³	10 ³ -10 ⁴	10 ⁴ -10 ⁵	10 ⁵ -10 ⁶	10 ⁶ -10 ⁷	10 ⁷ -10 ⁸	Mode	Average (95% CI) ^a
Cattle	104/474	–	21.9 (18.2–25.7)	369	80	2	10	5	1	0	1	4×10 ²	3×10 ⁴ (2×10 ² –8×10 ⁴)
Sheep	97/292	–	24.9 (20.6–29.2)	291	52	5	13	12	8	1	6	2×10 ⁵	2×10 ⁵ (7×10 ⁴ –4×10 ⁵)
Pig	27/101	–	26.7 (18.1–35.3)	72	9	4	5	5	4	1	0	3×10 ⁴	3×10 ⁴ (3×10 ³ –7×10 ⁴)
Bird ^b	11/34	16/80	23.7 (15.9–31.5)	86	17	2	3	2	3	0	0	8×10 ³	7×10 ³ (1×10 ³ –2×10 ⁴)
Duck	1/3	11/43	26.1 (13.4–38.8)	31	10	0	2	0	0	2	0	2×10 ⁵	4×10 ⁵ (5×10 ⁴ –1×10 ⁶)
Goose	4/12	13/56	25.0 (14.7–35.3)	49	13	1	2	0	0	2	0	2×10 ⁵	2×10 ⁵ (1×10 ² –4×10 ⁵)
Gull	1/1	47/215	22.2 (16.7–27.8)	165	38	2	3	2	2	2	1	2×10 ⁴	6×10 ⁴ (4×10 ³ –2×10 ⁵)
Pigeon	2/13	69/242	27.8 (22.3–33.3)	181	58	1	5	3	4	1	1	2×10 ⁵	2×10 ⁵ (3×10 ³ –4×10 ⁵)
Poultry	21/51	3/7	41.4 (28.7–54.1)	33	20	0	4	0	0	0	0	1×10 ²	2×10 ² (5×10 ¹ –4×10 ²)
Cat	0/6	2/38	4.5 (0–10.7)										
Dog	0/11	1/66	1.3 (0–2.8)										
Swan	–	4/39	10.3 (1–19.8)										
Other ^c	8/36	1/14	18.0 (0.07–28.6)										

^a Confidence interval calculated by the bootstrap method.

^b Bird denotes samples of avian origin but unknown species.

^c Other includes crow (1), deer (2), donkey (1), ferret (1), fly (3), fox (1), goat (4), hedgehog (1), horse (12), jackdaw (1), llama (1), pheasant (8), rabbit (5), rook (4), sparrow (1), starling (2), swallow (1), and toad (1). The *Campylobacter*-positive isolates were crow (1), horse (1), jackdaw (1), pheasant (5), and starling (1).
CI, confidence intervals.

Table 2Comparisons of *Campylobacter* Prevalence Among Hosts

Comparison	OR	95% CI	p
Cattle–poultry	0.40	0.23–0.70	0.047
Cattle–dog	21.36	2.94–155.50	<0.001
Sheep–dog	25.25	3.46–183.99	<0.001
Pigs–dog	27.73	3.67–209.34	<0.001
Birds–dog	23.59	3.13–177.72	<0.001
Duck–dog	26.28	3.35–214.65	0.002
Goose–dog	25.33	3.27–196.35	<0.001
Gull–dog	21.71	2.94–166.25	<0.001
Pigeon–cat	8.10	1.91–34.36	0.018
Pigeon–dog	29.33	4.00–214.92	<0.001
Poultry–cat	14.28	3.27–67.22	<0.001
Poultry–dog	53.65	6.97–412.94	<0.001
Poultry–swan	6.18	1.94–19.68	0.005

OR is defined as the ratio of the odds of a *Campylobacter*-positive isolate occurring in a given host divided by the odds of a positive isolate occurring in a second host. 95% CI, the table only shows comparisons that are statistically significant using Fisher's exact test after sequential Bonferroni correction for multiple comparisons ($n=78$).

Table 3

Statistically Significant Pairwise Comparisons of *Campylobacter* Diversity Between Animal Sources Determined by Rarefaction at the Level of Clonal Complex and Sequence Type

CC		ST	
<i>Comparison</i>	<i>p-Value</i>	<i>Comparison</i>	<i>p-Value</i>
Sheep<birds	0.0144		
Sheep<geese	0.0360		
Pigs<birds	0.0216		
Pigs<geese	0.0396		
		Pigs>pigeons	0.0036
Birds>pigeons	0.0036	Birds>pigeons	0.0036
Geese>pigeons	0.0108		
		Pigeons<poultry	0.0036

The table denotes which animal source has greater diversity of *Campylobacter* and associated *p*-value after Bonferroni correction ($n=36$). The *p*-values are determined by a randomization test.

CC, clonal complex; ST, sequence type.

Table 4

Genetic Distances (Manly, 1985) Within *Campylobacter* Reservoirs Calculated at the Clonal Complex, Sequence Type, and Allele Level

Level	Group	Sheep	Pigs	Birds	Ducks	Geese	Gulls	Pigeon	Poultry
CC	Cattle	0.389	0.913	0.754	0.890	0.971	0.821	0.940	0.660
	Sheep		0.679	0.771	0.907	0.961	0.861	0.965	0.469
	Pig			1.000	1.000	1.000	0.959	1.000	0.708
	Bird				0.756	0.848	0.959	0.846	0.719
	Duck					0.882	0.855	0.851	0.812
	Goose						0.959	0.897	0.895
	Gull							0.895	0.918
	Pigeon								0.888
ST	Cattle	0.527	1.000	0.814	0.981	0.981	0.945	0.967	0.825
	Sheep		0.965	0.848	0.990	0.989	0.938	0.976	0.729
	Pig			1.000	1.000	1.000	0.980	1.000	0.963
	Bird				0.859	0.902	0.958	0.904	0.884
	Duck					0.882	0.958	0.933	0.958
	Goose						0.958	0.921	0.958
	Gull							0.910	0.927
	Pigeon								0.963
Allele	Cattle	0.323	0.886	0.693	0.869	0.854	0.817	0.784	0.568
	Sheep		0.688	0.722	0.886	0.859	0.853	0.846	0.441
	Pig			0.995	1.000	0.966	0.961	1.000	0.699
	Bird				0.713	0.701	0.823	0.801	0.662
	Duck					0.749	0.846	0.863	0.821
	Goose						0.856	0.855	0.819
	Gull							0.823	0.856
	Pigeon								0.823

The figures in bold represent that the differences between the groups are significant at the $p < 0.05$ level following sequential Bonferroni correction ($n=36$). p -Values are determined by a randomization test.

Table 5

Correct Assignment to Host by Structure at the Level of Clonal Complex, Sequence Type, and Allele

Source	CC	ST	Allele
Cattle	30.0 (28.7–31.3)	27.3 (25.8–28.7)	65.2 (64.2–66.2)
Sheep	26.8 (25.5–28.1)	27.6 (26.1–29.1)	40.2 (39.1–41.2)
Pig	26.8 (24.4–29.4)	18.4 (15.9–21.1)	94.6 (93.6–95.7)
Bird	13.8 (11.9–15.7)	12.8 (10.8–15.1)	30.4 (28.7–32.2)
Duck	13.6 (10.9–16.4)	14.4 (11.1–18.2)	40.9 (38.4–43.2)
Goose	16.5 (13.9–19.1)	14.9 (12.0–17.8)	58.4 (56.1–60.5)
Gull	44.3 (42.7–46.0)	31.2 (29.2–33.2)	64.3 (63.5–65.1)
Pigeon	66.1 (64.8–67.3)	60.1 (58.5–61.7)	77.5 (77.1–77.8)
Poultry	12.5 (10.5–14.5)	12.7 (10.4–15.1)	20.9 (19.7–22.1)
Average	27.8	24.4	54.7