Genetic Diversity and Antimicrobial Susceptibility of *Campylobacter jejuni* Isolates Associated with Sheep Abortion in the United States and Great Britain

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*Campylobacter* infection is a leading cause of ovine abortion worldwide. Historically, genetically diverse *Campylobacter fetus* and *Campylobacter jejuni* strains have been implicated in such infections, but since 2003 a highly pathogenic, tetracycline-resistant *C. jejuni* clone (named SA) has become the predominant cause of sheep abortions in the United States. Whether clone SA was present in earlier U.S. abortion isolates (before 2000) and is associated with sheep abortions outside the United States are unknown. Here, we analyzed 54 *C. jejuni* isolates collected from U.S. sheep abortions at different time periods and compared them with 42 *C. jejuni* isolates associated with sheep abortion during 2002 to 2008 in Great Britain, using multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), and array-based comparative genomic hybridization (CGH). Although clone SA (ST-8) was present in the early U.S. isolates, it was not as tetracycline resistant (19% versus 100%) or predominant (66% versus 91%) as it was in the late U.S. isolates. In contrast, *C. jejuni* isolates from Great Britain were genetically diverse, comprising 19 STs and lacking ST-8. PFGE and CGH analyses of representative strains further confirmed the population structure of the abortion isolates. Notably, the Great Britain isolates were essentially susceptible to most tested antibiotics, including tetracycline, while the late U.S. isolates were universally resistant to this antibiotic, which could be explained by the common use of tetracyclines for control of sheep abortions in the United States but not in Great Britain. These results suggest that the dominance of clone SA in sheep abortions is unique to the United States, and the use of tetracyclines may have facilitated selection of this highly pathogenic clone.
(10). Moreover, studies, using a pregnant guinea pig model (14) demonstrated that clone SA was highly abortifacient compared to other C. jejuni strains, indicating the evolution of increased virulence in this clone. Recently, we reported that clone SA has emerged as a zoonotic pathogen, causing human gastroenteritis linked mainly to the consumption of raw milk (13). The genome of a representative of this clone, C. jejuni IA3902, has now been sequenced (15).

To date, limited information was available on the genetic diversity and antimicrobial susceptibilities of Campylobacter isolates from ovine abortion in countries other than the United States. The only published exception to this was New Zealand, where C. fetus continues to be a major cause of Campylobacter-associated abortion in sheep. Nevertheless, in New Zealand Campylobacter ovine abortion isolates (both C. fetus and C. jejuni) from different farms during different lambing seasons were found to be of multiple genotypes (7, 16, 17) suggesting that, at least in New Zealand, no single Campylobacter clone was dominant as a cause of ruminant abortion.

In England and Wales from 1997 to 2008, clinical samples (fetuses or placenta) from ruminant abortions (cattle, sheep, and goats) submitted to the Veterinary Laboratories Agency (now named the Animal Health and Veterinary Laboratories Agency [AHVLA], Surrey, England) were routinely cultured for Campylobacter. All Campylobacter isolates recovered were speciated and subspeciated, where relevant, and catalogued. In addition, abortion-associated isolates were submitted from Scotland for speciation. Thus, this constitutes a unique collection of isolates from Great Britain (England, Wales, and Scotland) for comparative studies.

The current study was undertaken to determine the genetic diversity and antimicrobial susceptibility of representative sheep abortion C. jejuni isolates from Great Britain and the United States in an effort to enhance our knowledge of the overall epidemiology and ecology of C. jejuni populations in ovine abortion worldwide and, in particular, to determine whether C. jejuni clone SA is present in ruminant abortions outside the United States. This information is critically needed for development and implementation of effective control measures in sheep flocks. In addition, C. jejuni isolates from sheep abortions that occurred before (pre-2000) the clone SA became epidemic in the United States (post-2003) were characterized in order to provide clues to the evolution of clone SA. The results indicate significant differences in the geographically and temporally separated populations. Further studies suggest that these differences may be associated with evolutionary selection pressures exerted by variations in antimicrobial use in veterinary medicine.

MATERIALS AND METHODS

Collection of C. jejuni isolates from sheep abortion. In this study, C. jejuni isolates (n = 54) derived from sheep abortion cases in the United States were comprised of two different temporal collections: the first set included isolated (n = 21) cultured from ovine abortions that occurred from 1991 to 2000 in California (n = 8) and Idaho (n = 13) (here referred to as the early U.S. isolates). The second set of isolates (n = 33) were recovered from sheep abortions from 2008 to 2011 from cases in Colorado (n = 2), Iowa (n = 27), and North Dakota (n = 4) (here referred to as the late U.S. isolates). All of the isolates were obtained from respective veterinary diagnostic laboratories that processed the submissions. In addition, data for 92 C. jejuni isolates from ovine abortions in the United States that occurred during 2003 to 2007 (also considered part of the late U.S. isolates) from a previously published investigation (10) were included for comparison, as indicated below.

The AHVLA has a collection of 924 stored Campylobacter ruminant abortion isolates from Great Britain from cases from 1997 to 2008. All isolates have been identified to the species/subspecies level by using standard biochemical and molecular methods (18). The majority of these isolates (66%) are C. fetus subsp. fetus, but 147 (~160) are C. jejuni. For this study, 42 of these C. jejuni isolates were selected as representative of cases of ovine abortion throughout Great Britain from 2002 to 2008.

MLST and phylogenetic analysis. MLST was performed following the method originally developed for C. jejuni by Dingle et al. (19). The primer sets for amplification and sequencing of the seven housekeeping genes were used, and the PCRs were performed as described on the C. jejuni MLST website (http://pubmlst.org/campylobacter/), which was developed by Keith Jolley and Man-Suen Chan (20). Allelic numbers were assigned to the isolates by performing BLAST searches for the assembled sequences using the single-locus query function. Sequence types were assigned using the allelic profile query function in the MLST database. Sequences that were identical to existing alleles in the MLST database were assigned the corresponding allele numbers. Novel sequences were assigned new allele numbers and sequence types (STs) within the MLST database.

Clonal genealogy of STs was estimated using a model-based approach with the ClonalFrame computer package for determining bacterial microevolution (21). This model calculates clonal relationships with improved accuracy compared with standard phylogenetic inference techniques for recombining bacteria, such as C. jejuni. It distinguishes point mutations from imported chromosomal recombination events, the source of the majority of allelic polymorphisms. The model has been used successfully to distinguish clades within Campylobacter species and can describe the relationships between genotypes (21). Nucleotide sequences of seven-locus STs were concatenated, and the program was run with 50,000 burn-in iterations followed by 50,000 data collection iterations. The tree required 75% consensus for inference of relatedness.

Genetic distance analysis. Pairwise comparisons of the genetic distances between each of the C. jejuni populations of interest (geographic and temporal) were calculated using the standardized genetic distances (d_I) as described previously (12, 22). When d_I is 1, there are no genotypes in common, and when d_I is 0, the two bacterial populations have the same distribution of genotypes. Genetic distance was determined at the level of ST, clonal complex (CC), and allele (for the allelic level, the right-hand side of the equation was modified to include the sum of the contribution across all seven MLST alleles, which was then divided by seven). The genetic distance between each pair of groups was tested for significance in the Visual Basic Application within Excel (VBAE) by comparing this distance with a distribution of 10,000 distances obtained by randomizing the data without replacement.

PFGE typing. PFGE analysis of the macrorestriction fragment patterns of genomic DNA using Smal and HpaI enzymes was performed following the Centers for Disease Control and Prevention (CDC) standardized PulseNet protocol for C. jejuni (23) and as described elsewhere (13). As the main purpose of performing PFGE was to further confirm the clonal relatedness of isolates having the same STs, a detailed restriction pattern-based genetic clustering was not carried out.

Array-based comparative genomic hybridization (CGH). 70-Mer oligo arrays were synthesized by JCVI (http://pfgrc.jcvi.org) or MYcroarray according to the complete genome of C. jejuni IA3902 (15). Genomic DNA was extracted by using the Promega genomic DNA purification kit (Madison, WI). DNA labeling, hybridization, and washing were performed following the standard protocol from JCVI or MYcroarray. Genomic DNA from each isolate (test DNA) was mixed with an equal amount of genomic DNA of strain IA3902 (reference DNA), and the mixture was hybridized onto two replicate slides by using a dye swap strategy. Microarray slides were scanned at 532-nm (Cy3) and 635-nm (Cy5) wavelengths using a GenePix 4100A microarray scanner (Molecular
Genotypes of Abortifacient Campylobacter from Sheep

RESULTS

Genotyping and phylogeny of *C. jejuni* isolates from sheep abortions. MLST analysis was performed on a total of 188 isolates derived from sheep abortions in the United States (446) and Great Britain (42). Overall, 28 STs were identified (Fig. 1). Twenty-three of these STs were clustered into 10 CCs. The remaining 5 STs were singletons (STs that could not be assigned to any CC). Three of these singletons were of newly identified STs (4841, 4842, and 4843; all from Great Britain), while the remaining two (ST-2165 from Great Britain and ST-441 from the United States) were previously reported. There was only one novel sequence type (ST-5189) within the U.S. strain collection. Fifteen STs were found only once in our entire abortion collection. accounting for 87.6% (128 of 146) of the isolates. Twenty-three of these STs were clustered into 10 CCs. Overall, 28 STs were identified (Fig. 1). Twenty-three of these STs were clustered into 10 CCs. The remaining 5 STs were singletons (STs that could not be assigned to any CC). Three of these singletons were of newly identified STs (4841, 4842, and 4843; all from Great Britain), while the remaining two (ST-2165 from Great Britain and ST-441 from the United States) were previously reported. There was only one novel sequence type (ST-5189) within the U.S. strain collection. Fifteen STs were found only once in our entire abortion collection. accounting for 87.6% (128 of 146) of the isolates.

The presence of the *tet*(O) gene (the main determinant of tetracycline resistance in *C. jejuni*) was determined by PCR as described previously (10). In *Campylobacter*, *tet*(O) is usually located on conjugative plasmids (such as pTet), although it can also be inserted in the chromosome. To determine the location of *tet*(O) (plasmid or chromosome), new primer sets were designed and used in a PCR. The genome sequence of strain IA3902 (a clone SA isolate) indicates that *tet*(O) is located in the chromosome within the CJSAN191 locus (corresponding to CJS1203 in the genome of NCTC11168). To ascertain whether the *tet*(O) gene was located in this chromosomal region, primer pairs tet-O-F2 (5′-CCT GGC GTA TCT ATA ATG TTG ACT-3′) and CJS191-R1 (5′-ATA AGG GCC TAA ATG ATCTGGA-3′) were used to generate an amplicon size of approximately 2,000 bp. In contrast, to determine if *tet*(O) was located on the pTet plasmid, primer sets tet-O-F1 (5′-TAG CCG TAT AGA TAA GGT TCG-3′) and cpp6-R1 (5′-CTG TGC ATA AAA TCA TAG AAT-3′), designed based on the pTet sequence (26), were employed to generate an amplicon size of about 3,500 bp.

Microarray data accession number. The microarray sequence data obtained from the CGH analysis were submitted to the GEO database and assigned the accession number GSE54860.

FIG 1 ClonalFrame phylogenetic tree for the 7-gene MLST data set, displaying the clonal relationship among the STs of 188 *C. jejuni* isolates from sheep abortions. The x axis is time in coalescent units. STs found among the Great Britain (GB) collection, those of the U.S. collection, and STs found among both collections are shown. Singletons (STs that could not be assigned to a CC) are denoted by UA. The number of isolates within each ST is shown in parentheses. Devices, Sunnyvale, CA) at 5-μm resolution. Fluorescence intensities of each spot were extracted using the GenePixPro 7.0 program (Molecular Devices). Analysis of the microarray data was conducted as follows: (i) after local background correction, the fluorescence intensity at each wavelength was log2 transformed and normalized using locally weighted linear regression (LOWESS) within the statistical software R (http://www.r-project.org); (ii) genes with a signal intensity ratio (test/reference) less than 0.6 in both replicate slides were considered divergent between the test genome and the reference genome. Data were submitted to the GEO database (see below). Divergent or conserved genes were translated to a binary code (1 for divergent, 0 for conserved) and analyzed with Cluster 3.0 and visualized using TreeView (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm).

Antimicrobial susceptibility testing and PCR screening for *tet*(O). The MICs of nine antibiotics were determined using a standard broth microdilution method as recommended by CLSI and National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) (10, 13). Commercially available Sensititre plates (Trek Diagnostic Systems, Cleveland, Ohio) were used for antimicrobial susceptibility testing. For all plates, *C. jejuni* ATCC 33560 was used as a quality control strain.

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50, 239, 607, 982, and 5189 (Fig. 1 and 2A and B). Apart from ST-8, there was little overlap in STs between the early and the late U.S. strains, except the second most predominant ST in the early U.S. isolates, ST-50, was detected only in 2004 (Fig. 2A and B). Table 1 shows the genetic distances between the two temporal collections, which indicate substantial genetic diversity between isolates from the early and the late U.S. collections at the ST level, but this difference was not statistically significant.

The 42 sheep abortion isolates from Great Britain were assigned to 19 STs and 8 CCs (4 STs were singletons) (Fig. 1 and 2C). The most common sequence type was ST-227 (19%; 8 isolates), followed by ST-206 (14.2%; 6 isolates), ST-270 (9.5%; 4 isolates), ST-19 and ST-61 (7.1% each; 3 isolates), and STs 42, 50, 137, and 1517 (4.7% each; 2 isolates). STs 21, 45, 52, 262, 432, 2165, 3153, 4841, 4842, and 4843 were each represented by a single isolate. These 19 STs appeared to occur sporadically during the years 2002 to 2008, and there were no obvious predominant STs within the Great Britain collection. The predominant ST within the U.S. collection, ST-8 (clone SA), was not found within the Great Britain strains investigated. However, ST 21, 42, 45, and 50 were present in both the U.S. and the Great Britain strain collections (Fig. 1 and 2). The observed differences in population structures between the U.S. and the Great Britain C. jejuni isolates from sheep abortions was supported by the genetic distance analysis (Table 1).

PFGE confirmed the differences in the U.S. and Great Britain strains populations observed by MLST. As previously reported (10), all ST-8 isolates from the U.S. sheep abortions were basically represented by two closely related KpnI subtypes and a single SmaI type. These PFGE types persisted over the time periods studied (results not shown). Interestingly, strains of some STs (ST-21, ST-42, and ST-45), despite having the same MLST, had very distinct PFGE patterns in the U.S. compared with Great Britain collections. In contrast, isolates of ST-50 were indistinguishable by PFGE regardless of the country of origin (results not shown).

Array-based CGH analysis of C. jejuni isolates from sheep abortions. Because the MLST only considered diversity in 7 genes, a CGH approach was adopted to investigate gene divergence across the whole genome. Seventeen representative strains, with distinct STs and PFGE profiles, were selected. This strain set included 4 early U.S. isolates, 9 late U.S. isolates, and 4 isolates representing the most common STs within the Great Britain strains (Table 2). Strain IA3902, representing clone SA, was used as the reference genome (GenBank accession numbers CP001876.1 for the chromosome and CP001877.1 for the pVir plasmid). Among all the strains investigated, 258 of the chromosomal genes di-

TABLE 1 Standardized pairwise genetic distances at the level of CC, ST, and allele between geographic and temporal groups of 188 C. jejuni abortion isolates in the United States and Great Britain

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Genetic distance ($d_i$)</th>
<th>$p^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUS vs GB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.8337</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ST</td>
<td>0.928</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Allele</td>
<td>0.5025</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LUS vs GB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.8095</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ST</td>
<td>0.9759</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Allele</td>
<td>0.5485</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>US vs GB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.8162</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ST</td>
<td>0.9456</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Allele</td>
<td>0.6701</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EUS vs LUS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.0587</td>
<td>0.409</td>
</tr>
<tr>
<td>ST</td>
<td>0.3252</td>
<td>0.0404</td>
</tr>
<tr>
<td>Allele</td>
<td>0.0188</td>
<td>0.0199</td>
</tr>
</tbody>
</table>

$^a$ EUS, early U.S. collection; LUS, late U.S. collection; GB, Great Britain collection. $^b$ $P$ values are raw; those in bold were statistically significant after Bonferroni correction ($n = 21$).
verged from those in the reference genome (Fig. 3; see also Data Set S1 in the supplemental material). These divergent genes were found to be functionally enriched in the Clusters of Orthologous Group (COG) categories of cell wall/membrane biogenesis, general function prediction only, and "not in COGs" (Fig. 4). A dendrogram based on the clustering of the divergent genes showed that all of the ST-8 genomes were closely related and had very similar gene content (Fig. 3). The U.S. isolate ND9 clustered together with the ST-8 genomes, despite having a different sequence type (ST-239). It is notable that both ST-8 and ST-239 belong to CC21.

A close examination of the distribution of the variable genes in the genomes revealed that most of them were present in clusters or variable regions (VRs), with the largest cluster comprising 30 consecutive genes (capsule biosynthesis locus; cjsa_1345-cjsa_1375). Overall, 74.8% (193/258) of the variable genes also had divergent neighbors. Moreover, 12 VRs (VR1 to -12), containing more than 5 consecutive genes, accounted for 58.5% (151/258) of the divergent chromosomal genes (Fig. 3; see also Data Set S1 in the supplemental material). These VRs encode genes involved in iron transport (VR1, cjsa_0167-cjsa_0171), zinc transport (VR2, cjsa_0238-cjsa_0242), the pantothenate and biotin biosynthesis pathway and molybdenum transport (VR3, cjsa_0269-cjsa_0274), altronate hydrolysis and fusose transport (VR4, cjsa_0450-cjsa_0461), phosphate regulation and iron uptake (VR6, cjsa_0690-cjsa_0714), glutamine binding (VR7, cjsa_0770-cjsa_0774), lipooligosaccharide biosynthesis (VR9, cjsa_1074-cjsa_1087), flagellar modification and O-linked glycosylation (VR10, cjsa_1234-cjsa_1276), capsular biosynthesis (VR11, cjsa_1345-cjsa_1375), DNA restriction/modification (VR12, cjsa_1465-cjsa_1473), and unknown function (VR5, cjsa_0532-cjsa_0536; VR8, cjsa_0912-cjsa_0916). All of the large VRs, except VR10, were identified to be conserved among the non-ST-8 genomes only. VR10, encoding flagellar modification and the O-linked glycosylation locus, was also divergent in strain ID15 (an early U.S. ST-8 isolate) compared with the rest of the ST-8 genomes. Besides the large VRs, small highly variable chromosomal loci were also identified among the tested genomes. Five loci (cjsa_0032-cjsa_0033, encoding a type II restriction/modification enzyme and a putative cytoplasmic protein, cjsa_130, encoding the McrBC restriction endonuclease system, cjsa_0192-cjsa_0193, encoding a hypothetical protein and tet(O), cjsa_0585-cjsa_0586, encoding hypothetical proteins, cjsa_0982, encoding mur(O)) were highly divergent among more than 50% of the tested genomes. Interestingly, the pVir plasmid (present in IA3902) was absent from all the isolates tested regardless of their sequence type and country of origin (Fig. 3). The heterogeneity within the genomes with respect to pVir carriage was further confirmed by PCR (data not shown).

### Antimicrobial susceptibility of the C. jejuni isolates.

The MICs at which the growth of 50% and 90% of the C. jejuni isolates from sheep abortions in Great Britain and the United States (n = 41 and 54, respectively) were inhibited and the percentages of resistance isolate for each antimicrobial drug are summarized in Table 3. Of 41 Great Britain isolates tested, 7 (17.1%) were resistant to nalidixic acid, 4 (9.76%) were resistant to clindamycin, 2 (4.88%) were resistant to tetracycline, and 1 (2.44%) was resistant to azithromycin. None of the Great Britain isolates showed any resistance to ciprofloxacin, erythromycin, florfenicol, gentamicin, or telithromycin. In comparison, all of the U.S. isolates were susceptible to clindamycin and florfenicol but had low levels of resistance (less than 5%) to azithromycin, ciprofloxacin, gentamicin, nalidixic acid, and telithromycin (Table 3). In contrast to the Great Britain isolates, 68.5% (37 of 54 total) of the U.S. isolates were resistant to tetracycline, with an MIC$_{90}$ of $>64$ µg/ml. There was also a dramatic increase in resistance to tetracycline between the early U.S. and late U.S. isolates (19% compared with 100%, respectively) (Table 3). All of the tetracycline-resistant isolates (n = 4) within the early U.S. strain collection were of ST-8, i.e., none of the other STs present in the early U.S. group (STs 21, 50, and 441) were resistant to tetracycline (see below).

### Characterization of tetracycline resistance in C. jejuni isolates.

By aggregating the data from this study with data from a previous study (10), a collection of tetracycline-resistant C. jejuni isolates from sheep abortions was obtained. This collection included 123 from the United States and 2 from Great Britain. The presence of the tetracycline resistance gene, tet(O), and its location (chromosome or plasmid) were determined for 99 of the 123 tet

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racycline-resistant U.S. isolates and both of the Great Britain isolates by PCR (Table 4). The tet(O) gene was detected in 96% (95/99) of the U.S. isolates, of which 92.6% (88/95) had it within cjsa_0191 locus in the chromosome and 7.3% (7/95) carried it on the pTet plasmid. All 4 of the tetracycline-resistant strains (all ST-8) from the early U.S. collection, but only 3 of the 91 late U.S. strains, had tet(O) on pTet (Table 4). Interestingly, one of the Great Britain isolates also carried the tet(O) gene within the cjsa_0191 locus, while the other isolate appeared to have it at an undetermined chromosomal locus. A detailed description of tet(O) presence and location by country of origin, year of isolation, and MLST is presented in Table 4.

**DISCUSSION**

Bacteria of the genus *Campylobacter* are associated with several disease presentations (2, 27–30). There are 27 species of *Campylobacter*, and the species *C. fetus* has been historically recognized as associated with abortion, especially in sheep and cattle (1, 6, 31, 32). However, it is known that the thermophilic species *C. jejuni* and *C. coli* can also cause abortion (5, 33). Recently in the U.S., one clone of *C. jejuni* (clone SA) has become predominant in *Campylobacter* isolates recovered from ovine abortions (10). The reason for and the geographical extent of this disease shift are as yet unknown. There are few other national collections of *Campylobacter* isolates from ovine abortions, but in Great Britain such isolates have been collected at the AHVLA for over a decade. We have now undertaken a comparison of U.S. and Great Britain strains from similar timelines by using the genotypic approaches of PFGE, MLST, and array-based CGH. All three methods were used, because they have different levels of sensitivity. MLST is the least sensitive method for typing, as it is dependent on only seven housekeeping genes (19). PFGE is dependent on point mutations in *C. jejuni* the plasticity of the genome is renown and minor changes can falsely indicate strain differences (34). Array-based CGH provides the most discriminatory power utilizing all the genomic detail of the bacterium. The use of these three methods sequentially, particularly the array-based GCH (Fig. 3), confirmed the genetic identities and clonal natures of the clone SA strains. Moreover, these techniques demonstrated that, unlike in the United States, where the population of ovine strains has become increasingly genotypically homogeneous (i.e., predominantly clone SA), the Great Britain strain population remains heterogeneous (Fig. 2). Interestingly, the Great Britain strains were even significantly different (P ≤ 0.0001 in the genetic distance analysis) from the early U.S. strains, i.e., before the predominance of clone SA (Table 1). The reason for this is unclear but suggests a historical geographical separation in the bacterial populations. Whether the differences
seen between strains from the United States and Great Britain are consistent worldwide is yet to be investigated.

MLST was developed as a technique to determine evolutionary trends. The population structure of C. jejuni and its close relative, C. coli, is now well established, with nearly 30,000 isolates included in the database (http://pubmlst.org/campylobacter/). A detailed analysis was undertaken of the MLST genotypes of the ovine abortion strains relative to the population distribution of all C. jejuni strains. Interestingly, the STs of the ovine abortion strains in the joint Great Britain and early U.S. collections is largely representative of the strains from sheep/sheep feces submitted to the MLST C. jejuni/C. coli database (41/11005 385 strains as of 27 January 2014) and similar to those described in other ruminant studies in Great Britain (12). This indicates that, until recently, there has been no particular selection of strains with specific virulence properties for ovine abortion, and that the organism, which is normally a commensal of the ovine intestinal tract, inherently has all the phenotypic properties enabling it to translocate across the intestinal epithelium and infect the placenta of naive (previously unexposed) sheep and cause fetal mortality. The definitive evidence for this would come from a genomic comparison of clone SA and other strains isolated from sheep but that are unassociated with abortion.

The absence of evidence for specific virulence properties of C. jejuni essential for ovine abortion suggests that the rapid expansion of clone SA in affected sheep in the United States is due to selection pressure independent of the host. The presence of tetracycline resistance in this clone suggests that the veterinary use of this antimicrobial has provided the required selection pressure. This hypothesis is supported by antimicrobial susceptibility analysis of the strain collections, which showed that tetracycline resistance was very low in C. jejuni isolates from Great Britain (4/11021 5%) and, to a lesser extent, in the early U.S. collection (19%), but most recently all of the ovine abortion C. jejuni isolates have displayed resistance to this antibiotic (Table 3). At the same time, there has been a general shift in the location of the tet(O) gene apparently responsible for this resistance, from the plasmid, pTet, to the chromosome (Table 4). Tetracyclines are commonly used in sheep in the United States, particularly for the control of ovine abortion storms associated with Campylobacter (35).

![Diagram of Gene Enrichment Analysis](https://jcm.asm.org/...)

**FIG 4** Gene enrichment analysis of the divergent genes (identified via CGH) among 18 C. jejuni isolates from sheep abortions. The fraction of genes in each COG category is shown on the x axis. COG categories that were significantly enriched (P < 0.0001, Z-test) are indicated by an asterisk.

### TABLE 3 Antimicrobial susceptibilities of 54 U.S. and 41 Great Britain C. jejuni isolates from sheep abortions

<table>
<thead>
<tr>
<th>Agent</th>
<th>GB isolates* (n = 41)</th>
<th>U.S. isolates (n = 54)</th>
<th>Early U.S. isolates (n = 21)</th>
<th>Late U.S. isolates (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>% resistant</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0.12</td>
<td>0.12</td>
<td>2.44</td>
<td>0.06</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.12</td>
<td>0.25</td>
<td>0</td>
<td>0.12</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.12</td>
<td>0.25</td>
<td>9.76</td>
<td>0.12</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.5</td>
<td>1</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>Florfenicol</td>
<td>1</td>
<td>2</td>
<td>2.44</td>
<td>1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>8</td>
<td>32</td>
<td>17.1</td>
<td>8</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>0.5</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.25</td>
<td>2</td>
<td>4.88</td>
<td>64</td>
</tr>
</tbody>
</table>

*Isolates from the Great Britain collection.
The divergent genes among the C. jejuni isolates from sheep abortion were found to be enriched in the COG functional categories of cell wall/membrane biogenesis, general function prediction, and function unknown proteins (Fig. 4). Many of these variable regions involve genes associated with the lipooligosaccharide biosynthesis locus (VR9, cjsa_1074-cjsa_1087), flagellar modification and O-linked glycosylation locus (VR10, cjsa_1234-cjsa_1276), and capsular biosynthesis locus (VR11, cjsa_1345-cjsa_1375). Previous studies indicated that the pathogenicity of C. jejuni can be significantly influenced by changes in the genomic content of such regions. Variations in the flagellar locus can lead to differences, in both the flagellin protein backbone and post-translational modifications of flagellin (42). Diversity in the capsular locus can result in the presence of many different capsule types (43), and variation in LOS biosynthesis loci can produce different surface lipooligosaccharides (43, 44). It is generally accepted that the LOS, capsule, and flagella are important in the C. jejuni pathogenic mechanisms of campylobacteriosis (42), but whether differences in these bacterial structures would confer properties that enable ovine abortion is unknown. One possibility is the development of antigenic diversity (43), which could facilitate host immune evasion. Recent studies using multi-omics approaches and a guinea pig abortion model to compare clone SA with C. jejuni strain NCTC11168 also implicated such genetic and phenotypic characteristics in the generation of a highly successful abortifacient C. jejuni strain (15). The genomic sequencing of multiple ovine abortion strains is now being undertaken in an attempt to identify those genes that both confer the phenotypic properties enabling C. jejuni to overcome innate immune responses and cause the systemic maternal infection resulting in fetal abortion; such changes have enabled clone SA to outcompete all other C. jejuni strains to become predominant as a cause of ovine abortion in the United States.

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