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Genetic Relationships among Reptilian and Mammalian *Campylobacter fetus* Strains Determined by Multilocus Sequence Typing[▽]

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Reptile *Campylobacter fetus* isolates and closely related strains causing human disease were characterized by multilocus sequence typing. They shared ~90% nucleotide sequence identity with classical mammalian *C. fetus*, and there was evidence of recombination among members of these two groups. The reptile group represents a possible separate genomospecies capable of infecting humans.

Campylobacter fetus is a human and animal pathogen which can be divided into two subspecies: subsp. *fetus* and subsp. *venerealis* (16). *C. fetus* subsp. *fetus* has a wide host range and causes abortions in sheep and cattle; *C. fetus* subsp. *venerealis* is host restricted, being isolated specifically from the bovine genital tract, and it causes fertility problems in cattle (5). *C. fetus* is an opportunistic pathogen in humans, particularly affecting severely immunocompromised patients. Initially, the bacterium can cause gastroenteritis; then, bacteremia can lead to septicemia and disseminated infections (1, 8). These two subspecies of mammalian *C. fetus* are referred to subsequently as "classical *C. fetus*."

A multilocus sequence typing (MLST) scheme has been developed for classical *C. fetus* (http://pubmlst.org/cfetus/) and used to genotype 140 isolates from humans and animals (14). The data showed that classical *C. fetus* is genetically homogeneous and clonal. *C. fetus* has also been isolated from reptiles (7), and DNA hybridization and nucleotide sequence data indicate that these reptile *C. fetus* isolates are genetically distinct from classical *C. fetus* (12). Reptile-like *C. fetus* strains have also been isolated from cases of human disease (11). In the present study, both the reptile and the human reptile-like strains are referred to collectively as "reptile *C. fetus* strains."

The MLST scheme for classical *C. fetus* was modified in this study to allow typing of reptile *C. fetus* (Table 1) and comparisons within and among *Campylobacter* species. The MLST method for classical *C. fetus* (15) was modified as follows. First, the annealing temperature of the PCR amplification was reduced to 47°C. Second, one of the oligonucleotide primers used to amplify the *glyA* locus (glyA2) was replaced with glyS4, 5′-AGGTGATTATCCGTTCCATCGC-3′, derived from the *C. jejuni* sequence. New allele and ST numbers were assigned, and the data were deposited at http://pubmlst.org/cfetus/. Data analysis was performed using the programs MEGA (http://www.megasoftware.net/) (9) and ClonalFrame (3). ClonalFrame is a model-based method for using multilocus sequence data to infer the clonal relationships of bacteria and the chromosomal position of homologous recombination events that disrupt a clonal pattern of inheritance.

Five reptile-derived and six human-derived (two from the same patient) reptile *C. fetus* strains were typed (Table 1). Allele sequences and therefore all sequence types (STs) differed from those described previously for classical *C. fetus*. A total of seven new STs were identified among the 11 reptile strains (Table 1). Compared to the classical strains, the reptile group was more variable, also confirmed by the presence of three STs in one turtle. The data were used to investigate the relationships among all known *C. fetus* STs ($n = 30$) including classical mammalian strains and reptile strains of both reptile and human origin. The classical STs differed by only 27 of 3,312 nucleotides (0.82%) and 7 of 1,104 amino acids (0.63%). Greater nucleotide sequence variation was detected within the reptile *C. fetus* STs, with 87 of 3,312 (2.62%) variable nucleotide sites and nine (0.82%) amino acid substitutions. When the classical and reptile groups were compared, there were 281 of 3,312 (8.48%) variable nucleotide sites and 15 of 1,104 (1.35%) amino acid substitutions, demonstrating that the two groups were distinct, each showing a high level of clonality. Strains with ST-16 and ST-26 were not included in this analysis, as they contained "imported alleles" and represented possible recombinants, as described below.

The genetic relationships among the classical and reptile *C. fetus* strains were investigated further. The nucleotide se-

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TABLE 1. MLST data for *C. fetus* reptile isolates recovered from humans and reptiles

Strain	Source	Location	Yr isolated	ST	Allele no.:							Reference
					aspA	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*	
03-427	Human[a]	NY, USA	2003	15	5	6	6	4	6	6	6	11
03-445	Human[a]	NY, USA	2003	15	5	6	6	4	6	6	6	11
05-018	Human[b]	NY, USA	2005	15	5	6	6	4	6	6	6	Unpublished
D6659	Human[b]	MA, USA	2005	15	5	6	6	4	6	6	6	Unpublished
D6683	Human[b]	MA, USA	2005	15	5	6	6	4	6	6	6	Unpublished
91-2	Human[b]	Denver, CO, USA	1991	30	5	6	6	10	6	6	5	Unpublished
85-387	Turtle	CA, USA	1984	16	3	3	6	6	4	5	5	7
85-388	Turtle	CA, USA	1984	17	4	4	6	5	5	5	5	7
85-389	Turtle	CA, USA	1984	18	6	3	6	5	5	5	5	7
CF78	Skink[c]	London Zoo, UK	2003	26	10	3	6	8	7	9	5	Unpublished
SP3	Snake[c]	UK	2006	27	11	8	6	9	7	10	5	Unpublished

[a] Isolates from the same patient with a 37-day interval.
[b] Isolates from human clinically ill patients confirmed as reptile *C. fetus* strains using *sap* insertion PCR (10).
[c] Isolates confirmed as reptile *C. fetus* strains using *sap* insertion PCR (10).

quences of the alleles comprising the 30 STs were concatenated, and a consensus tree was constructed using ClonalFrame (3) and viewed using MEGA (9) (Fig. 1). The tree revealed two distinct clusters comprising (i) the classical *C. fetus* strains and (ii) the reptile *C. fetus* strains. The *C. fetus* subsp. *venerealis* strains formed a subgroup within the classical *C. fetus* group, as shown previously using a neighbor-joining tree (Fig. 1) (15).

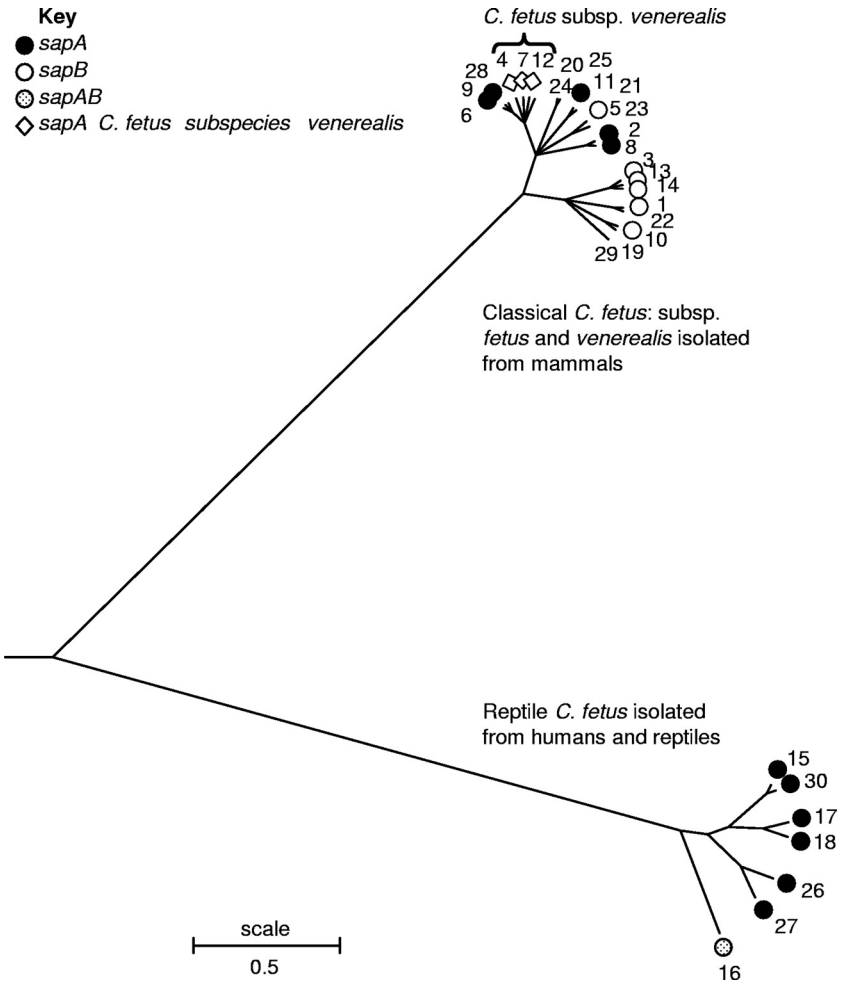

FIG. 1. Consensus tree (Newick tree) constructed using ClonalFrame (2) and viewed using MEGA (9) to show the two distinct groups formed by classical mammalian and reptile *C. fetus*. *sap* types associated with the STs are indicated. Input sequences comprised the concatenated sequences of the seven MLST loci.

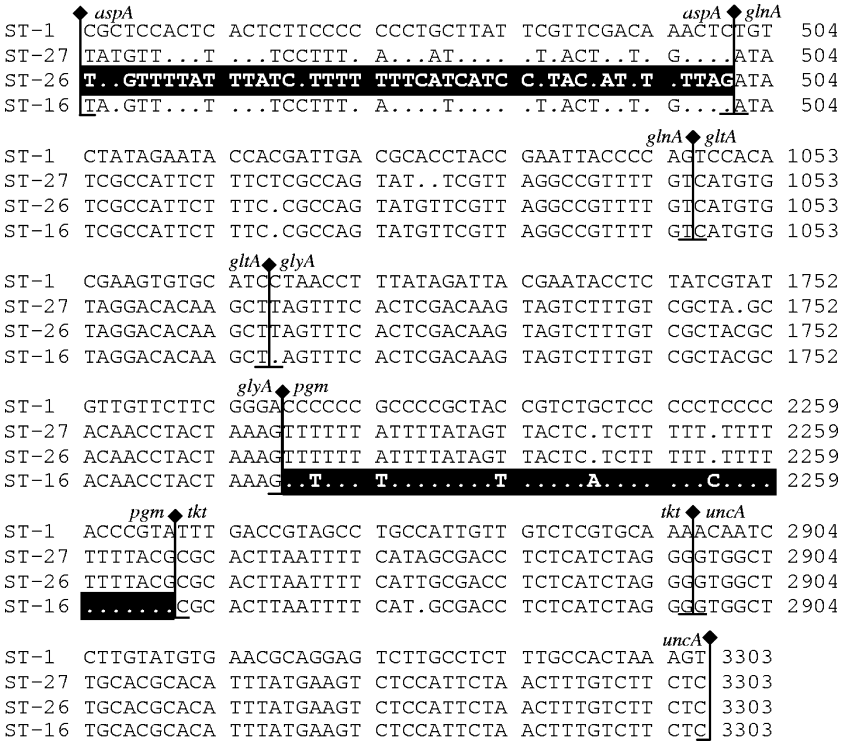

FIG. 2. Nucleotide sequence alignment of concatenated STs showing the variable sites only. Dots indicate identity to ST-1. This illustrates the relationship between classical mammalian *C. fetus*, represented by ST-1, and reptile *C. fetus*, represented by ST-27. The confirmed recombinant reptile *C. fetus* strain, ST-16, has a high level of sequence identity with classical mammalian *C. fetus* in the *pgm* locus, shown by black shading. The possible recombinant ST-26 reptile *C. fetus* strain has a sequence divergent from those of reptile *C. fetus* ST-27 and ST-16 in the *aspA* locus, indicated by black shading.

The divergence in nucleotide sequence between reptile *C. fetus* and classical *C. fetus* (8.64%) is comparable to the divergence between *C. jejuni* and *C. coli* within these housekeeping gene loci. For example, the central genotypes of the most common *C. jejuni* and *C. coli* clonal complexes, ST-21 and ST-828 (4), are 13.5% divergent. In contrast, there were only 15 of 1,104 (1.35%) amino acid changes between classical and reptile *C. fetus*, compared to 5.16% for *C. jejuni* and *C. coli* (ST-21 and ST-828). This may indicate that the two *C. fetus* groups share a more recent common ancestor than *C. jejuni* and *C. coli*.

Sequence alignments of the variable sites in the 30 concatenated ST nucleotide sequences (four representatives shown in Fig. 2) indicated that ST-16 and ST-26 were potential recombinants, each containing an apparently imported "foreign" sequence at one of seven loci: *aspA* for ST-26 and *pgm* for ST-16. The program ClonalFrame confirmed that ST-16 was a recombinant between reptile *C. fetus* and a strain very closely related to classical *C. fetus*. This observation suggests that reptile and mammal *C. fetus* strains may have mixed at some point in an individual host. The closest relative of the "imported allele" in ST-26 in GenBank was classical *C. fetus*, with which the imported allele shares 92% identity, indicating that its precise species of origin has yet to be identified.

The correlation of *sap* type with the two *C. fetus* groups was investigated (Fig. 1). *sap* type is determined by surface layer proteins, an orderly paracrystalline array and major virulence factor for host colonization and prevention of complement-mediated immune responses (2, 13). The *sap* genes can be rearranged on the chromosome, contributing to antigenic diversity of the S layer and inhibiting immune detection (14). The *sap* type of the possible recombinant ST-16 was unique, being *sapAB*, an observation supporting the hypothesis that it may have undergone a major recombinational event.

The *C. fetus* genome sequence (subsp. *fetus* strain 82-40; human isolate TIGR project ID 16293) was examined within the region of the recombinant loci. Both *pgm* and *aspA* are located near genes encoding either flagellin or Sap proteins (http://msc.tigr.org/campy/campylobacter_fetus_subsp_fetus_82_40/index.shtml), major antigens in *C. fetus* subject to selective pressure (2, 17). These genes are known to be prone to chromosomal rearrangements in campylobacters (6, 14, 17). Also, *pgm* is located about 2 kb from a putative site-specific recombinase (SSR) from the phage integrase family. This provides further evidence of the potential for these genomic regions to be involved in recombination events.

In conclusion, reptile *C. fetus* strains of both human and reptile origin are genetically distinct from classical mammalian *C. fetus*. The confirmed recombinant ST-16, identified in a turtle isolate, contained both reptile *C. fetus* and classical *C. fetus*-like sequences. Although they clustered together (Fig. 1), none of the STs of the reptile *C. fetus* strains isolated from humans were identical to those that have been isolated thus far from reptiles. Since the number of strains studied was low, it is not yet clear whether transmission of these strains occurs among reptiles and humans or whether the two hosts represent

separate reservoirs. This reptile *C. fetus* cluster may represent a separate genomospecies.

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