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Distinct Genotypes of Human and Canine Isolates of *Campylobacter upsaliensis* Determined by 16S rRNA Gene Typing and Plasmid Profiling

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The utility of combined 16S rRNA (*rrs*) gene restriction fragment length polymorphism and plasmid profiles to differentiate between and within *Campylobacter upsaliensis* of human and canine origin was examined. Fourteen distinct *rrs* gene restriction fragment length polymorphs consisting of bands sized between 1.9 and 4.8 kb were observed. The copy number of the 16S rRNA gene was three in most strains of *C. upsaliensis*. Plasmids were found in almost 60% of the strains; ranging in size from 1.5 to 100 kb, they gave 15 distinct plasmid profiles. All isolates from humans contained one or more plasmids, as did strains isolated from dogs with sporadic diarrhea. The two commonest 16S ribotypes were divided into eight and nine subgroups by plasmid profiling. The genotyping of canine isolates from three veterinary surveys detected both multiple infections and reinfection of dogs. Except for one, each of the isolates from humans constituted a single and unique 16S ribotype, and these more frequently carried plasmids than did canine strains. Ribotypes of human strains were not found among canine isolates. These results suggest that host-specific genotypic differences may exist among strains of *C. upsaliensis*, for example, intraspecific clones or clone complexes pathogenic for humans.

The prevalence of catalase-negative thermotolerant campylobacters, which were subsequently named *Campylobacter upsaliensis* (20), in human disease has probably been underestimated because of their sensitivity to antibiotics used in the selective isolation media developed for *Campylobacter jejuni* (7). *C. upsaliensis* has, however, been isolated successfully from diarrheal stools by filtration methods (6, 25), and the organism has also been cultured directly from human blood (13). In certain hospital surveys *C. upsaliensis* accounted for more than 20% of all *Campylobacter* isolates (4). It has consequently become recognized as an emerging pathogen associated with human gastroenteritis and bacteremia (18). As the first reported isolations of *C. upsaliensis* (then described as a catalase-negative or weak campylobacter) were from feces of healthy as well as of diarrheic dogs (21), it is of increasing interest whether such pet animals constitute a significant reservoir of zoonotic infection of humans (8).

Molecular typing of *Campylobacter* spp. has been addressed principally by rDNA hybridization analysis in genomic Southern blots, also referred to as ribotyping (15, 16, 19). Recently the analysis of species of other genera such as *Helicobacter* and *Salmonella* (5, 14, 22) employed an intragenic probe amplified by PCR from the 16S RNA (*rrs*) gene of the organism of interest, which generates simplified ribotypes directly related to gene copy numbers.

Plasmid profile typing has been shown to be relevant in the study of *C. upsaliensis* (4, 7, 12, 17). Owen and Hernandez (17) detected extrachromosomal DNAs in 87% of strains, observing fifteen different plasmid profiles and an association of plasmid profile with geographical area of strain origin. Goossens et al. (7) detected plasmids in 90% of isolates from humans of *C. upsaliensis* and found that 60% of these strains shared a single plasmid profile.

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In this study we compare the relative utilities of (16S rRNA gene) ribotyping and plasmid profiling for identifying strain genealogies and for detailed epidemiological studies of *C. upsaliensis* from human and canine infections.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Strains of *C. upsaliensis* are listed in Table 1 and were grown as previously described (15). The first source group was geographically diverse and included human fecal and blood isolates. The second source group comprised canine strains isolated in Sweden during the early 1980s and included the type strain of *C. upsaliensis*. The remaining three source groups comprised canine isolates from three veterinary surveys of *C. upsaliensis* in Switzerland. Subset 3 consisted of isolates from individual healthy pet dogs housed at a boarding kennel. Subset 4 consisted of isolates from pups of seven distinct breeds, raised separately at different kennels. Subset 5 consisted of isolates from sick but unrelated dogs, i.e., from sporadic cases of canine diarrhea. DNA of all strains failed to hybridize in dot blots with the probe pCH1, which is specific for the related species *Campylobacter helveticus* (23), but did react positively with a total genomic DNA probe from *C. upsaliensis* NCTC 11540.

Fresh overnight subcultures were used for plasmid analysis, whereas genomic DNA was prepared from plates incubated for 48 to 72 h. Strains were preserved at −70°C on glass beads in nutrient broth no. 2 (CM67; Oxoid) containing 10% (vol/vol) glycerol.

Genomic DNA extraction, probe preparation, and hybridization. Genomic DNA was extracted by the method of Wilson (26), with additional purification by a second extraction with cetyltrimethylammonium bromide-NaCl and dialysis of the sample against Tris-EDTA (10:1 mM) buffer in a microdialysis unit (Gibco-BRL). Genomic Southern blots were prepared and hybridized as previously described (22).

TABLE 1. Bacterial strains used in this study

Strain	Country of origin	Source (breed)	16S ribotype	Plasmid band(s) (kb)
Subset 1				
NCTC 11840	United Kingdom	human feces	*Cu*-RXII	
NCTC 11926	Australia	human feces	*Cu*-RIII	100, 53, 7, 4.7
NCTC 11927	Australia	human feces	*Cu*-RIII	100, 53, 7, 4.7
NCTC 12183	South Africa	human blood	*Cu*-RIII	100, 7, 4.7
NCTC 12184	South Africa	human blood	*Cu*-RIII	100, 53, 7, 4.7
NCTC 12206	Belgium	human feces	*Cu*-RIII	100, 8.7
NCTC 12264	Belgium	human feces	*Cu*-RIII	100, 53, 7, 4.7
A771/92	South Africa	human blood	*Cu*-RIII	100, 53, 7, 4.7
A772/92	South Africa	human blood	*Cu*-RIII	100, 53, 7, 4.7
A773/92	South Africa	human blood	*Cu*-RIII	94, 47, 7, 4.7, 1.5
A774/92	South Africa	human blood	*Cu*-RIII	94, 47, 7, 4.7, 1.5
A775/92	South Africa	human blood	*Cu*-RIII	94, 47, 7, 4.7, 1.5
A776/92	South Africa	human blood	*Cu*-RIII	94, 47, 7, 4.7, 1.5
Subset 2				
NCTC 11540	Sweden	dog feces	*Cu*-RII	56
NCTC 11541	Sweden	dog feces	*Cu*-RXI	
A1000/92	Sweden	dog feces	*Cu*-RI	
A1001/92	Sweden	dog feces	*Cu*-RI	
A1002/92	Sweden	dog feces	*Cu*-RIX	
A1004/92	Sweden	dog feces	*Cu*-RI	
A1005/92	Sweden	dog feces	*Cu*-RI	
A1006/92	Sweden	dog feces	*Cu*-RVII	66, 47
A1007/92	Sweden	dog feces	*Cu*-RIX	
A1008/92	Sweden	dog feces	*Cu*-RIV	
A1009/92	Sweden	dog feces	*Cu*-RIV	
A1010/92	Sweden	dog feces	*Cu*-RX	
A1011/92	Sweden	dog feces	*Cu*-RXIII	
A1012/92	Sweden	dog feces	*Cu*-RIV	
A1013/92	Sweden	dog feces	*Cu*-RIV	
A1014/92	Sweden	dog feces	*Cu*-RI	
A1015/92	Sweden	dog feces	*Cu*-RIV	
A1016/92	Sweden	dog feces	*Cu*-RII	
A1017/92	Sweden	dog feces	*Cu*-RII	47
A1018/92	Sweden	dog feces	*Cu*-RIV	100
A1019/92	Sweden	dog feces	*Cu*-RII	
A1020/92	Sweden	dog feces	*Cu*-RVII	47
Subset 3				
C19	Switzerland	kennelled dog	*Cu*-RII	78
C21	Switzerland	kennelled dog	*Cu*-RII	78, 47
C27	Switzerland	kennelled dog	*Cu*-RII	47
C35	Switzerland	kennelled dog	*Cu*-RXIV	47
C39	Switzerland	kennelled dog	*Cu*-RI	
C43	Switzerland	kennelled dog	*Cu*-RIV	
C51	Switzerland	kennelled dog	*Cu*-RIV	
C159	Switzerland	kennelled dog	*Cu*-RVIII	
C196	Switzerland	kennelled dog	*Cu*-RII	66
C433	Switzerland	kennelled dog	*Cu*-RVIII	47
C435	Switzerland	kennelled dog	*Cu*-RII	78
C449	Switzerland	kennelled dog	*Cu*-RII	47
C450	Switzerland	kennelled dog	*Cu*-RIV	
C451	Switzerland	kennelled dog	*Cu*-RII	66
C453	Switzerland	kennelled dog	*Cu*-RIV	
C454	Switzerland	kennelled dog	*Cu*-RII	47
C460	Switzerland	kennelled dog	*Cu*-RI	
C462	Switzerland	kennelled dog	*Cu*-RII	
C549	Switzerland	kennelled dog	*Cu*-RVII	94
C57	Switzerland	kennelled dog	*Cu*-RIV	94, 47
C447	Switzerland	kennelled dog	*Cu*-RII	47
C32	Switzerland	kennelled dog	*Cu*-RII	66, 47
C441	Switzerland	kennelled dog	*Cu*-RI	
Subset 4				
C104	Switzerland	pup 1 (A)	*Cu*-RI	
C107	Switzerland	pup 4 (A)	*Cu*-RI	47
C108	Switzerland	pup 5 (A)	*Cu*-RI	
C109	Switzerland	pup 7 (A)	*Cu*-RII	47, 23, 9

Continued on following page

TABLE 1—*Continued*

Strain	Country of origin	Source (breed)	16S ribotype	Plasmid band(s) (kb)
C238	Switzerland	pup 3 (A)	*Cu*-RI	
C239	Switzerland	pup 4 (A)	*Cu*-RI	
C242	Switzerland	pup 8 (A)	*Cu*-RI	
C335	Switzerland	pup 7 (A)	*Cu*-RI	94, 47
C306	Switzerland	pup 11 (B)	*Cu*-RI	78
C307	Switzerland	pup 12 (B)	*Cu*-RI	78
C498	Switzerland	pup 12 (B)	*Cu*-RII	66
C114	Switzerland	pup 14 (C)	*Cu*-RI	47, 23, 9
C162	Switzerland	pup 14 (C)	*Cu*-RI	
C294	Switzerland	pup 26 (D)	*Cu*-RI	
C370	Switzerland	pup 27 (D)	*Cu*-RI	
C368	Switzerland	pup 25 (D)	*Cu*-RI	47
C369	Switzerland	pup 26 (D)	*Cu*-RI	
C365	Switzerland	pup 21 (D)	*Cu*-RI	47
C610	Switzerland	pup 19 (D)	*Cu*-RI	66, 47
C611	Switzerland	pup 20 (D)	*Cu*-RI	66, 47
C612	Switzerland	pup 21 (D)	*Cu*-RI	66, 47
C613	Switzerland	pup 22 (D)	*Cu*-RI	66, 47
C618	Switzerland	pup 27 (D)	*Cu*-RI	66, 47
C619	Switzerland	pup 28 (D)	*Cu*-RI	66, 47
C426	Switzerland	pup 31 (E)	*Cu*-RII	47
C562	Switzerland	pup 36 (F)	*Cu*-RVI	
C563	Switzerland	pup 37 (F)	*Cu*-RVI	
C565	Switzerland	pup 39 (F)	*Cu*-RVI	
C566	Switzerland	pup 40 (F)	*Cu*-RVI	
C428	Switzerland	pup 43 (G)	*Cu*-RV	47
C429	Switzerland	pup 44 (G)	*Cu*-RV	47
C431	Switzerland	pup 46 (G)	*Cu*-RV	47
C578	Switzerland	pup 46 (G)	*Cu*-RV	47
Subset 5				
E471-90	Switzerland	sporadic diarrhea	*Cu*-RI	66
E544-90	Switzerland	sporadic diarrhea	*Cu*-RI	47
E711-90	Switzerland	sporadic diarrhea	*Cu*-RI	78, 47
E869-90	Switzerland	sporadic diarrhea	*Cu*-RI	66, 47
E1660-90	Switzerland	sporadic diarrhea	*Cu*-RI	66, 47
E1685A-90	Switzerland	sporadic diarrhea	*Cu*-RI	70
E1760A-90	Switzerland	sporadic diarrhea	*Cu*-RI	47

An *rrs* gene probe of either 1500 or 550 bp representing an almost full-length copy or an internal fragment of the genes was generated by PCR using the primer pairs 1510 and ε (11) and 1510 and U1 (2), respectively (see Fig. 1). The template was 100 ng of genomic *C. upsaliensis* NCTC 11540. PCR reactions and probe labelling were carried out as previously described (22).

Plasmid DNA extraction. Plasmid DNA was prepared by two methods in order to achieve a comprehensive profile (see Results). The first method was modified from that of Kado and Liu (9) so that cells were resuspended directly into 250 μl of 3% sodium dodecyl sulfate at pH 12.0 and incubated at 55°C for 20 min. An equal volume of phenol-chloroform was added and mixed in thoroughly, and the mixture was then centrifuged at 12,000 × *g* for 10 min. The second method used was a nonalkaline extraction method (1). Plasmid preparations were stored at 4°C and subjected to electrophoresis on 0.7% (wt/vol) agarose slab gels at 90 or 100 V for 2 h.

RESULTS

***rrs* gene RFLP patterns: general considerations.** The 1500-bp amplicon was used to probe *Hin*dIII-digested genomic DNA of 98 *C. upsaliensis* strains (Fig. 1). The sizes of homologous *Hin*dIII fragments are listed in Table 2. Fourteen distinct *rrs* restriction fragment length polymorphism (RFLP) patterns were observed. These polymorphs were composed of homologous fragments of 15 different molecular weights, 9 of which (Table 2 and Fig. 1) occurred in more than one RFLP pattern; these were termed "16S ribotypes". Ten such patterns designated by the prefix *Cu*-R were composed of three bands (90 of 98 strains), three patterns were composed of four bands, and one pattern was composed of two bands. The fourteen 16S ribotypes defined four major groups of strains, three minor groups containing 3 to 4 strains only and seven small groups with only one or two members (Table 2). The major 16S ribotypes were *Cu*-RI (37 of 98 strains) and *Cu*-RII (18 of 98 strains).

16S ribotypes of human isolates. Twelve strains in this subset (no. 1) of isolates had 16S ribotype *Cu*-RIII. This ribotype did not vary with country of origin and was not found in any canine isolate (see below). One human isolate (NCTC 11840) possessed a related 16S ribotype (*Cu*-RXII), in which the 3.1-kb band was replaced by one of 2.7 kb.

16S ribotypes of Swedish canine isolates. In the 22 isolates (Table 1, subset 2) from Swedish dogs, eight different 16S ribotypes were detected. Six strains had a 16S ribotype termed *Cu*-RIV, while five isolates had ribotype *Cu*-RI, the commonest one among all canine strains. All but one isolate exhibited profiles consisting of three homologous *Hin*dIII fragments. In strain A1010/92, the three bands of ribotype *Cu*-RI were accompanied by an additional *Hin*dIII band of 3.7 kb.

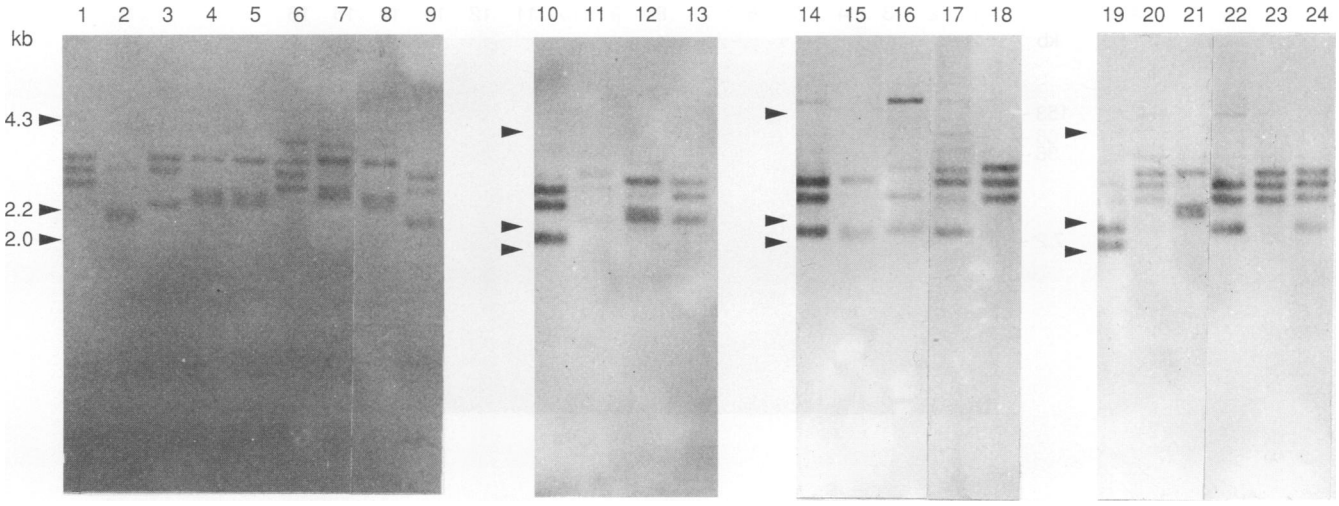

FIG. 1. 16S ribotypes of *C. upsaliensis*. Types were selected from four genomic Southern blots (*Hin*dIII digest) hybridized with biotinylated 16S rRNA gene probes. Since the electrophoretic migrations were different, molecular size markers (4.3, 2.2, and 2.0 kb) are indicated to the left of each blot by arrowheads. Ribotypes are shown in tracks as follows: *Cu*-RI, 1, 18, 20, and 23; *Cu*-RII, 10, 14, and 22; *Cu*-RIII, 13; *Cu*-RIV, 4 and 5; *Cu*-RV, 17; *Cu*-RVI, 16; *Cu*-RVII, 2; *Cu*-RVIII, 24; *Cu*-RIX, 3; *Cu*-RX, 6; *Cu*-RXI, 11; *Cu*-RXII, 12; *Cu*-RXIII, 7; and *Cu*-RXIV, 19.

Canine strains from Switzerland. Among 63 isolates from three veterinary studies, eight different 16S ribotypes were detected. The largest groups of isolates had ribotypes *Cu*-RI (32 of 63 strains) and *Cu*-RII (14 of 63 strains). In the 23 isolates (Table 1, subset 3) from unrelated dogs lodged at a single kennel, the predominant RFLP pattern was *Cu*-RII (11 of 23 strains). In isolates from the remaining 12 kennelled dogs, ribotypes *Cu*-RI, IV, VII, VIII and XIV were detected. In the two paired isolates (C57 with C447 and C32 with C441), ribotyping showed evidence of strain replacement over a 2-month period (Table 1).

Among the 32 strains (Table 1, subset 4) from healthy pups at seven different breed-specific kennels, four different ribotypes were detected, and the majority of strains (22 of 32) had ribotype *Cu*-RI. All pups with the exception of no. 14, which was infected at birth, were colonized at around 6 weeks of age, without developing any symptoms. Seven of eight isolates from the (six) breed A pups had ribotype *Cu*-RI, as did all 10 isolates from breed D pups. All isolates from pups of breeds F and G had ribotypes *Cu*-RVI and *Cu*-RV, respectively. The seven isolates (Table 1, subset 5) from diseased unrelated dogs with sporadic cases of diarrhea all had ribotype *Cu*-RI.

Apparent copy number of the *rrs* gene in *C. upsaliensis*. The 16S rRNA gene (GenBank accession no. L14628) of *C. upsaliensis* contains a *Hin*dIII site at base pair 72, and this is contained at the 5′ end of the 1.5-kb PCR amplicon used as described above as a general probe. A 550-bp amplicon which does not contain a *Hin*dIII site was generated with the same reverse primer (annealing at nucleotide [nt] 1510) and a forward primer (U1) annealing at nt 960. This probe was used to reexamine selected strains; it detected the same number and the same sizes of homologous bands as described above. Thus, 90 strains contained three homologous *Hin*dIII fragments sized between 2.1 and 3.3 kb, while seven strains contained four fragments and one strain contained fragments of 1.9 and 2.2 kb. Each gene (approximately 1.5 kb) is likely to be represented by one such *Hin*dIII band. The apparent gene

TABLE 2. 16S ribotypes observed in this study

16S Ribotype	*Hin*dIII fragment sizes (kb)	No. of strains[a]
Cu-RI	3.3, 3.0, 2.6	37
Cu-RII	3.0, 2.6, 2.2	18
Cu-RIII	3.4, 3.1, 2.6	12
Cu-RIV	3.3, 2.5, 2.4	11
Cu-RV	3.2, 3.0, 2.2	4
Cu-RVI	4.8, 3.3, 3.0, 2.2	4
Cu-RVII	3.1, 2.3, 2.4	3
Cu-RVIII	3.3, 3.0, 2.6, 2.1	2
Cu-RIX	3.3, 3.0, 2.1	2
Cu-RX	3.7, 3.3, 3.0, 2.6	1
Cu-RXI	3.4, 3.0, 2.6	1
Cu-RXII	3.4, 2.7, 2.6	1
Cu-RXIII	3.3, 2.6, 2.5	1
Cu-RXIV	2.2, 1.9	1

[a] Total number of strains, 98.

TABLE 3. Plasmid profiles obtained in this study

Plasmid profile	Plasmid size(s) (kb)	No. of strains[a]
PP0	none	44
PP1	47	17
PP2	66, 47	8
PP3	94, 47	5
PP4	100, 53, 7, 4.7	5
PP5	94, 47, 7, 4.7, 1.5	4
PP6	78	4
PP7	66	4
PP8	100, 7, 4.7	2
PP9	47, 23, 9	2
PP10	100	1
PP11	100, 8.7	1
PP12	94	1
PP13	78, 47	1
PP14	70	1
PP15	56	1

[a] Total number of strains, 98.

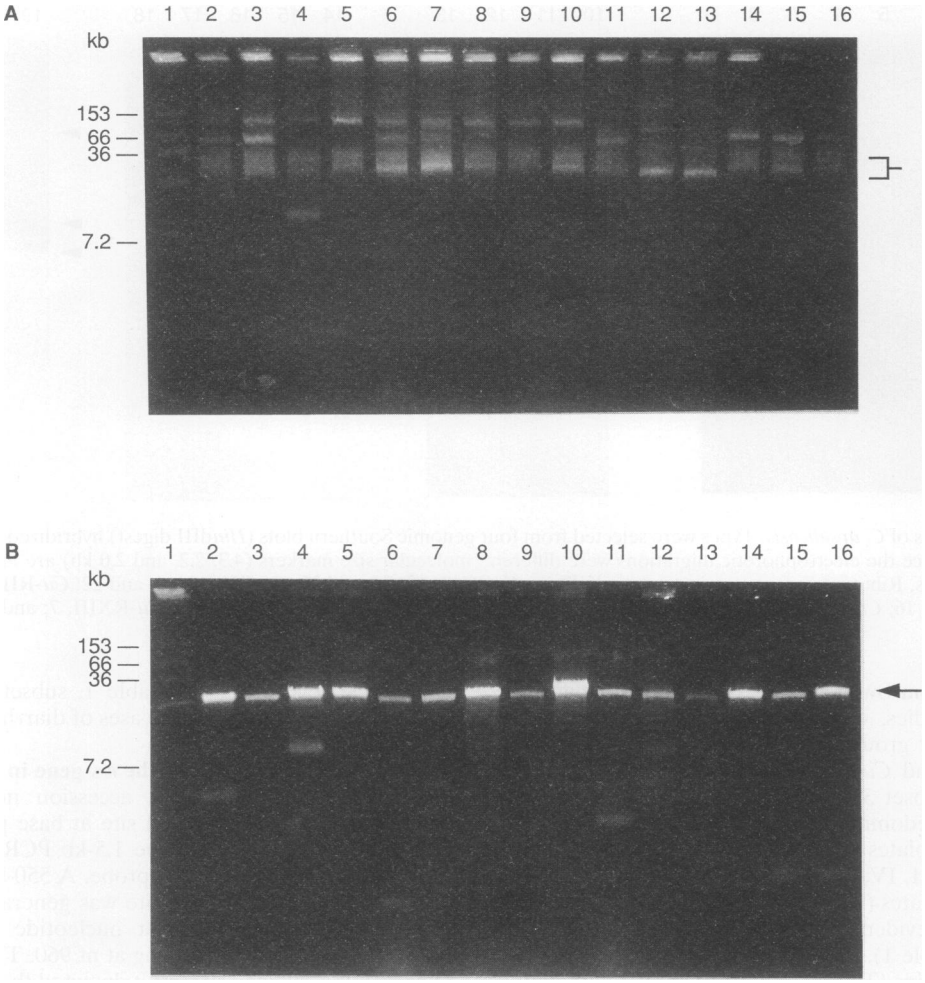

FIG. 2. Plasmid profiles of *C. upsaliensis*. (A) Agarose gel electrophoresis (100 V in 0.7% agarose) of plasmid profiles prepared by the method of Kado and Liu (9). Track 1 contains an *Escherichia coli* strain (39R861; NCTC 50192) with molecular size marker plasmids of 153, 66, 36, and 7.2 kb. Tracks 2 to 16 contain *C. upsaliensis* strains as follows: 2, NCTC 11926; 3, NCTC 12184; 4, NCTC 12206; 5, A1018/92; 6, A773/92; 7, C335; 8, C549; 9, E711-90; 10, C19; 11, E1685-90; 12, C32; 13, A1006/92; 14, 11540; 15, C109; and 16, A1019/92. Bracket at right indicates diffuse band of sheared chromosomal DNA seen with this method. (B) Agarose gel electrophoresis (90 V in 0.7% agarose) of the plasmid profiles prepared by the method of Alter and Subramanian (1). Sample order is as in panel A. Arrow at right indicates chromosomal DNA. Not all the small plasmids detected are easily visible.

copy number was, then, estimated to be three in more than 90% of *C. upsaliensis* strains but to be either two or four in certain strains.

Plasmid profiles: general considerations. During initial screening of the *C. upsaliensis* strains, certain plasmids proved sensitive to the alkaline treatment used in the analytical method of Kado and Liu (9). Although the pH of the lysis solution was lowered from 12.5 to 12.2, this failed to ensure efficient extraction of such plasmids. Nonalkaline plasmid extraction (1) was therefore used in order to recover these plasmids, and the results of the two methods (compare Fig. 2A and B) were combined to produce a composite plasmid profile (see explanation below).

Plasmid bands were detected in 54 of 98 (58%) of all *C. upsaliensis* isolates and ranged in size from 1.5 to 100 kb. Plasmid bands were present in a number of size combinations, allowing 15 plasmid-containing profiles (and one plasmid-free profile) to be defined (Table 3). Three or more plasmid bands were carried by 13 of 98 strains, and two were carried by 15 of 98 strains. No plasmid DNA was detected in more than 40% of strains. Other than this 40%, the most common profile consisted of a single 47-kb plasmid band, and one of the same size was found in 20 other strains with five separate profiles. The next most common plasmid bands in terms of molecular weight were sized at 66 kb, 94 kb, and 100 kb.

The thirteen human isolates of *C. upsaliensis* (subset 1, Table 1) carried two or more plasmid bands, consisting of a large 100- or 94-kb band and one to four smaller bands. The profile PP4 was found in six strains from Australia, South Africa, and Belgium. The profile PP5 was found in four strains isolated from pediatric blood cultures from South Africa (4).

Among the 22 canine strains from Sweden, only five carried plasmids (Table 1, subset 2). Of the 63 canine strains from Switzerland, more than 60% contained plasmid bands (Table 1, subsets 3 to 5). Most contained one or two plasmid bands sized at 47 or more kb. The most common profile (15 of 63) among these strains was PP1, consisting of a single 47-kb plasmid band. Nineteen isolates (Table 1, subset 4) from the

study with breeding dogs contained five different plasmid profiles. The plasmid bands found in 16 isolates (Table 1, subset 3) from the kennelled dogs formed five different profiles. The isolates (Table 1, subset 5) from animals with sporadic cases of diarrhea all carried plasmid bands, occurring in five different profiles. None of the plasmid-containing profiles found in the canine isolates were found in the human isolates.

DISCUSSION

Fourteen different *rrs* gene RFLPs, termed 16S ribotypes, were found among the strains of *C. upsaliensis*. They consisted of two to four *Hin*dIII bands of twelve different sizes, each readily recognizable, whose prevalence differed in the various subgroups of strains. Fifty-six percent of the strains could be assigned to two of these 16S ribotypes. A 16S rRNA gene copy number of three was detected for 90% of the *C. upsaliensis* strains in this study. This is consistent with the results of Taylor et al. (24) and Kim et al. (10) for *C. jejuni* and *Campylobacter coli*. One *C. upsaliensis* strain exhibited two *Hin*dIII bands; however, comigrating fragments cannot be excluded, and this need not imply a copy number of two such as was found in the related species *Helicobacter pylori* by Linton et al. (14) and Bukanov and Berg (3). There were, however, three 16S ribotypes (seven strains) consisting of four *Hin*dIII bands; this indicates that although most bacterial species are believed to have consistent copy numbers of the rRNA genes, *C. upsaliensis* strains may have variable copy numbers of this gene.

Plasmid profiling alone cannot be regarded as a primary typing option for *C. upsaliensis*, since only about half (42 of 98) of the strains, irrespective of the animal host, carried plasmids. This frequency is lower than that found in previous studies (7, 17), and the difference may be due to the fact that those studies were based on strains predominantly of human origin. Five of the 11 16S ribotypes did not contain plasmid-bearing strains. Seventeen strains of ribotype *Cu*-RI were plasmid free and could not be further subdivided. In cases in which plasmids were present, their profiles provided complementary subtyping schemes within the 16S ribotypes; for example, the two most abundant ribotypes could be subdivided into eight or nine subgroups each. Plasmid profiling was most useful for human isolates; all human isolates contained multiple plasmids, in five different combinations. There were several instances of chromosomal genetic diversity, as demonstrated by 16S ribotyping, among strains with the same plasmid profile. For example, a 47-kb plasmid occurred alone in six different 16S ribotype backgrounds, a 66-kb plasmid occurred in two ribotype backgrounds, and so forth. Conversely, some plasmid profiles were uniquely linked to chromosomal backgrounds, such as PP4 and PP5 to 16S ribotype *Cu*-RIII.

With respect to the veterinary implications of the canine studies, the following points can be made. In the kennel study, *Campylobacter* isolates (Table 1, subset 3) were studied in relation to the presence of dogs in the kennel. The dog population was constantly changing as new animals entered and others left. Some incoming animals were seen to have brought in new strains: e.g., a dog (no. 2738) brought in isolate C196 of 16S ribotype *Cu*-RII and PP7 and was the likely source of infection of another animal (no. 2750) which arrived later in the kennel. A question which was posed was whether there would be a few circulating strains in the kennel. This was not the case, since there was coexistence of a large number of different *C. upsaliensis* strains in the animals: the great majority of isolates obtained (on each of two occasions) had different genotypes. Not surprisingly, dogs frequently got reinfected but, interestingly, always without ill effect. This is important, since the majority of profiles from the healthy animals (Table 1, subset 3) were not distinguishable from those of strains isolated during episodes of illness (subset 5). In the breeding study (Table 1, subset 4), all pups were colonized at around 6 weeks of age—again, without developing symptoms. There were many instances of reinfection with a different strain (pup no. 4, pup no. 7, etc.), instances of multiple infection of a litter (breed A), and instances of genotypes which were breed specific (*Cu*-RVI and PP0 with breed F and *Cu*-RV and PP1 with breed G). All these results underline the opportunistic nature of most strains of *C. upsaliensis* as intestinal pathogens in dogs.

A striking feature of this study of *C. upsaliensis* was the genotypic distinction between strains from humans and those from dogs. All but one of the human isolates in this study had a conserved 16S ribotype which was not found in any of the canine isolates, and the distinct ribotype of the single plasmid-free human isolate was also not found among canine isolates. This is despite the fact that the origins of these human strains were geographically and culturally diverse, varying from aboriginal people in Australia to children in South Africa and patients in Europe. Human isolates also had a generally higher incidence of plasmids than did canine strains. The 16S ribotype data may be taken to indicate that clones or clone complexes within *C. upsaliensis* are specifically responsible for human disease. This deserves further investigation and should be investigated in relation to potential reservoirs of zoonotic infection by *C. upsaliensis*. Our general conclusion is that the combination of 16S ribotyping with plasmid profiling is very valuable for detailed epidemiological studies of *C. upsaliensis*. For the studies with dogs, many observations (e.g., of multiple infections or of rates of reinfection) could be made only in the light of typing evidence. Such genotypic typing methods are required to clarify the general status of *C. upsaliensis* in human or animal disease and its potential routes of zoonotic infection. Our longer-term goal is the establishment of a reproducible genotypic typing scheme for this emerging pathogen.

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