Campylobacter hyoilei Alderton et al. 1995 and *Campylobacter coli* Véron and Chatelain 1973 Are Subjective Synonyms

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The taxonomic affiliation of *Campylobacter hyoilei* was reevaluated by examining a variety of phenotypic and genotypic criteria. Whole-cell protein electrophoresis and a comparison of 66 phenotypic characters revealed that reference strains of *C. hyoilei* were indistinguishable from *Campylobacter coli* strains. These data were confirmed by a DNA-DNA hybridization level of 67% between the type strains of the two species. Several species-specific assays based on PCR amplification or probe hybridization further substantiated that *C. coli* strains and *C. hyoilei* strains are indistinguishable. It is therefore concluded that *C. hyoilei* and *C. coli* represent the same species and that the former name should be regarded as a junior synonym of the latter name.

The taxonomic structure and relationships of the family *Campylobacteraceae* have been unravelled by an integrated polyphasic approach (40, 41, 44, 49). As in many other lineages of the class *Proteobacteria*, results from rRNA homology experiments formed the framework used to revise the classification and nomenclature of campylobacters (17, 31, 36, 41, 44). Assignment of taxa to the genus *Campylobacter* and to the related genera *Arcobacter* and *Helicobacter* is primarily based on rRNA similarity data, in accordance with the present view of classification (namely, that natural relationships between bacteria [55] should be reflected where possible). Although definitely among the most valuable taxonomic tools, rRNA sequence analysis is not the appropriate method for resolving every problem in bacterial classification. The resolution of 16S rRNA sequence analysis for closely related species (organisms sharing more than 97% of their 16S rRNA sequences) has been shown to be low (5, 11, 35). For such organisms, DNA-DNA hybridization experiments are essential for species demarcation, and guidelines have been presented by an ad hoc committee (54). However, the interpretation of DNA-DNA hybridization results is often not straightforward as the threshold values for species delimitation vary among taxa and data obtained in different laboratories or with different hybridization methods often do not correlate (46).

In the present study, we evaluated the criteria used to assign 12 porcine isolates to the new species *Campylobacter hyoilei* (1). Remarkably, strains classified as *C. hyoilei* and *Campylobacter coli* were reported to produce exactly the same band patterns in several DNA probe hybridization assays, and the phenotypic data used to differentiate *C. hyoilei* from *C. coli* were at variance with other published data (1, 3, 27).

MATERIALS AND METHODS

Strains used. The strains used and their sources are listed in Table 1. Bacteriological purity was checked by plating and examining living and Gram-stained cells.

Polyacrylamide gel electrophoresis of whole-cell proteins. All of the strains examined were grown on Mueller-Hinton agar (catalog no. CM 337; Oxoid, Ltd.,

Basingstoke, United Kingdom) supplemented with 5% (vol/vol) horse blood and were incubated at 36 to 37°C in a microaerobic atmosphere containing approximately 5% O₂, 3.5% CO₂, 7.5% H₂, and 84% N₂.

Whole-cell protein extracts were prepared from three *C. hyoilei* strains and from type or reference strains of all *Campylobacter* species and of representative *Arcobacter* and *Helicobacter* species. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described previously (32). A densitometric analysis, normalization and interpolation of the protein profiles, and a numerical analysis were performed by using the GelCompar software package, version 4.0 (Applied Maths, Kortrijk, Belgium). The profiles were recorded and stored on a personal computer. The similarity between pairs of traces was expressed by the Pearson product moment correlation coefficient; data are presented below as percentages of similarity for convenience.

Phenotypic analysis. For phenotypic testing, nine strains were examined, six of which were type or reference strains. The strains examined were *C. hyoilei* CCUG 33450^T, CCUG 33769A, and CCUG 33770A, *C. coli* CCUG 11283^T, LMG 9799, CDC D112, CDC D126, and CDC D134, and *Campylobacter jejuni* subsp. *jejuni* CCUG 11284^T. The identities of *C. coli* CDC D112, CDC D126, and CDC D134 were established previously by quantitative DNA-DNA hybridization, and these strains exhibited 73 to 93% DNA homology to the *C. coli* type strain in reassessment experiments performed at both 50 and 65°C (18).

Strains were grown on 5% (vol/vol) calf blood agar for 3 days under microaerobic conditions, as described previously (24). A total of 66 phenotypic characters were determined for the *C. hyoilei* strains by using methods described previously (14, 19, 22–26). The test for nitrite reduction was amended; the base medium used was brain heart infusion broth (Difco Laboratories, Ltd., East Molesey, England) and not heart infusion broth (Difco). The remaining 65 tests comprise a comprehensive identification scheme for campylobacteria (27); two tests of this scheme, tests for growth at 30°C and on buffered charcoal-yeast medium, were not used. Previously described tests (1) and or tests identified in this study (see below) as being useful for differentiating *C. hyoilei* from *C. coli* were used to examine each of the 11 strains included in these experiments. These tests (Table 2) were performed on two separate occasions, and freshly prepared media were used to evaluate test reproducibility.

DNA-DNA hybridization experiments. The degree of DNA-DNA binding, expressed as a percentage, was determined spectrophotometrically by the initial renaturation rate method of De Ley et al. (10). Each value given below is the average of the values from at least two hybridization experiments. Values of DNA binding of 30% and less do not represent significant DNA homology. The total DNA concentration was about 39 µg/ml, and the optimal renaturation temperature in 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) was 63.0°C.

GTPase gene assay. DNA was isolated as described previously (12). The PCR mixtures (total volume, 100 µl) consisted of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM MgCl₂, 0.01% gelatin, and 0.1% Triton X-100. Deoxyribonucleotide triphosphates were used at a final concentration of 0.2 mM, and 0.25 U of Super Taq DNA polymerase (Sphaero Q, Leiden, The Netherlands), 10 ng of target DNA, and 50 pmol of primers were added to each reaction mixture. The primers, deduced from semiconserved GTP-binding sites G-1 and G-3, were used to amplify 156-bp fragments from various *Campylobacter* species, including *C. jejuni*, *C. coli*, *Campylobacter lari*, *Campylobacter upsaliensis*, *Campylobacter helveticus*, *Campylobacter fetus*, and *Campylobacter hyointestinalis* (51). Each PCR comprised 40 cycles consisting of denaturation for 1 min at 94°C, primer anneal-

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TABLE 1. Strains examined

Strain ^a	Other designation(s) ^a	Source
<i>Arcobacter cryaerophilus</i> LMG 7536 ^T	CCUG 17801 ^T	Aborted bovine fetus (Northern Ireland)
<i>Arcobacter nitrofigilis</i> LMG 7604 ^T	CCUG 12022 ^T	Roots of <i>Spartina alterniflora</i> (Canada)
<i>Bacteroides ureolyticus</i> LMG 6451 ^T	CCUG 7319 ^T	Amniotic fluid (Canada)
<i>Campylobacter coli</i> CCUG 11283 ^T	ATCC 33559 ^T , LMG 8847 ^T	Porcine feces (Belgium)
<i>Campylobacter coli</i> LMG 9799		Porcine intestine (Belgium)
<i>Campylobacter coli</i> CDC D112		Unknown
<i>Campylobacter coli</i> CDC D126		Unknown
<i>Campylobacter coli</i> CDC D134		Unknown
<i>Campylobacter concisus</i> LMG 7788 ^T	CCUG 13144 ^T	Human gingival sulcus (United States)
<i>Campylobacter curvus</i> LMG 7609 ^T	CCUG 13146 ^T	Human alveolar abscess
<i>Campylobacter fetus</i> subsp. <i>fetus</i> LMG 6442 ^T	CCUG 6823A ^T	Brain, sheep fetus
<i>Campylobacter fetus</i> subsp. <i>venerealis</i> LMG 6443 ^T	CCUG 538 ^T	Vaginal mucus, heifer (United Kingdom)
<i>Campylobacter gracilis</i> LMG 14731	CCUG 13143, FDC 406	Human periodontitis (United States)
<i>Campylobacter helveticus</i> LMG 12638 ^T	CCUG 12470 ^T , ATCC 51209 ^T	Feline feces (Switzerland)
<i>Campylobacter hyoilei</i> CCUG 33450 ^T	LMG 15882 ^T , RMIT 32A ^T	Porcine intestine (Australia)
<i>Campylobacter hyoilei</i> CCUG 33769A	LMG 15883, Alderton #1	Porcine intestine (Australia)
<i>Campylobacter hyoilei</i> CCUG 33770A	LMG 15884, Alderton #2	Porcine intestine (Australia)
<i>Campylobacter hyointestinalis</i> subsp. <i>hyointestinalis</i> LMG 7817 ^T	CCUG 14169 ^T	Porcine intestine (United States)
<i>Campylobacter hyointestinalis</i> subsp. <i>lawsonii</i> LMG 14432 ^T	P3-4 No. 15 ^T	Porcine stomach (United Kingdom)
<i>Campylobacter jejuni</i> subsp. <i>doylei</i> LMG 8843 ^T	NCTC 11951 ^T	Human feces (Australia)
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i> CCUG 11284 ^T	ATCC 33560 ^T , LMG 8841 ^T	Bovine feces
<i>Campylobacter lari</i> LMG 8846 ^T	NCTC 11352 ^T , ATCC 35221 ^T	Seagull intestine
<i>Campylobacter lari</i> LMG 17564		Shellfish (The Netherlands)
<i>Campylobacter lari</i> LMG 17558		Shellfish (The Netherlands)
<i>Campylobacter lari</i> PC 637		Human
<i>Campylobacter mucosalis</i> LMG 6448 ^T	CCUG 6822 ^T	Porcine intestine (United Kingdom)
<i>Campylobacter rectus</i> LMG 7613 ^T	FDC 371 ^T	Human periodontal pocket (United States)
<i>Campylobacter showae</i> LMG 12635 ^T	CCUG 30254 ^T	Human gingival crevice
<i>Campylobacter sputorum</i> LMG 7795 ^T	CCUG 9728 ^T	Human oral cavity
<i>Campylobacter upsaliensis</i> LMG 8850 ^T	NCTC 11541 ^T	Canine feces (Sweden)
<i>Helicobacter pylori</i> LMG 7539 ^T	CCUG 17874 ^T	Human stomach (Australia)

^a ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.; LMG, Laboratorium voor Microbiologie Gent Culture Collection, Universiteit Gent, Ghent, Belgium; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom; SVS, Statens Veterinære Serumlaboratorium, Copenhagen, Denmark.

ing for 1 min at 50°C, and chain extension for 1 min at 74°C. Prior to cycling, the samples were heated for 2 min at 94°C.

PCR products were separated on 2% agarose gels, stained with ethidium bromide, and transferred to Hybond N⁺ membranes (Amersham) by vacuum blotting. Southern blot hybridization was performed with [γ -³²P]ATP (Amersham)-labeled oligonucleotides by using standard procedures (34).

PCR products containing one biotinylated primer were sequenced as described by Hultman et al. (15).

glyA gene assays. Degenerate primers S1 (5'-AA[C/T] AAA TA[C/A] GC[A/T] GAA GG[A/T] TAT-3') and S2 (5'-ATG CAT [C/T]AA [A/T]GG [A/T]CC [A/T]CC TTG-3') were designed on the basis of a nucleotide analysis of the *C. jejuni* ATCC 43431 *glyA* gene (4) to amplify internal *glyA* fragments of approximately 640 bp from the genomic DNAs of the *C. jejuni*, *C. coli*, and *C. lari* type strains. Species-specific oligonucleotides were designed based on the sequences of these *glyA* fragments (2). By using the initial degenerate primers, additional

glyA fragments were amplified from genomic DNAs of the strains shown in Fig. 1, separated on a 1% agarose gel, and transferred onto a GeneScreen Plus nylon membrane (NEN Dupont). The different *Campylobacter* species were detected in two sets of Southern blot hybridizations; the species-specific oligonucleotides were radiolabeled and used as probes in one set, while the 640-bp *glyA* fragments from the type strains of *C. jejuni*, *C. coli*, and *C. lari* were radiolabeled and used as probes in the other set.

***C. jejuni*-*C. coli* multiplex PCR assay.** *C. hyoilei* CCUG 33450^T, CCUG 33769A, and CCUG 33770A and *C. coli* LMG 9799 were grown at 37°C in heart infusion broth (catalog no. 0038-17-7; Difco Laboratories, Detroit, Mich.) under microaerobic conditions. Five microliters of culture was mixed with 5 μ l of H₂O and kept for 10 min at 100°C. Samples were brought to a final volume of 50 μ l for PCR with a solution containing 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM MgCl₂, 0.01% gelatin, and each deoxyribonucleotide triphosphate at a concentration of 0.2 mM. Each reaction mixture also contained 1 U of *Thermus aquati-*

TABLE 2. Differential characteristics for the strains of *C. hyoilei*, *C. coli*, and *C. jejuni* subsp. *jejuni* examined

Strain	H ₂ S-TSI test	Nitrite reduction ^a	Alpha-hemolysis	Basic fuchsin test	5-Fluorouracil test
<i>C. hyoilei</i> CCUG 33450 ^T	+	—	+	I	—
<i>C. hyoilei</i> CCUG 33769A	+	—	+	I	—
<i>C. hyoilei</i> CCUG 33770A	—	—	+	+	—
<i>C. coli</i> CCUG 11283 ^T	+	—	—	I	+
<i>C. coli</i> LMG 9799	+	—	+	+	—
<i>C. coli</i> CDC D112	—	ND	+	+	—
<i>C. coli</i> CDC D126	—	ND	+	+	—
<i>C. coli</i> CDC D134	—	ND	+	+	+
<i>C. jejuni</i> CCUG 11284 ^T	—	—	+	+	+

^a All strains were found to reduce nitrate with the method used.

^b +, positive; —, negative; I, test irreproducible upon repeat testing; ND, not determined.

FIG. 1. Autoradiograph of the Southern hybridization membrane which was probed with the radiolabeled *C. coli* CCUG 11283^T *glyA* fragment. Strain CCUG 33450 is the *C. hyoilei* type strain, strains NCTC 11352^T, LMG 17558, LMG 17564, and PC 637 are *C. lari* strains, and strain CCUG 11284 is the *C. jejuni* subsp. *jejuni* type strain.

cus (Taq) DNA polymerase (Perkin-Elmer), 20 pmol of primer COL1 (5'-AG GCA AGG GAG CCT TTA ATC-3') (a *C. coli*-specific primer), 20 pmol of primer COL2 (5'-TAT CCC TAT CTA CAA ATT CGC-3') (a *C. coli*-specific primer), 20 pmol of primer JUN3 (5'-CA TCT TCC CTA GTC AAG CCT-3') (a *C. jejuni*-specific primer), and 20 pmol of primer JUN4 (5'-AAG ATA TGG CTC TAG CAA GAC-3') (a *C. jejuni*-specific primer) (primers were derived from randomly cloned DNA fragments selected for species specificity). All reactions were performed by using a touchdown protocol (33). Each PCR comprised an initial denaturation for 5 min at 94°C; 2 cycles consisting of denaturation for 1 min at 94°C, primer annealing for 1 min at 64°C, and chain extension for 1 min at 72°C; 2 cycles consisting of denaturation for 1 min at 94°C, primer annealing for 1 min at 62°C, and chain extension for 1 min at 72°C; 2 cycles consisting of denaturation for 1 min at 94°C, primer annealing for 1 min at 60°C, and chain extension for 1 min at 72°C; 2 cycles consisting of denaturation for 1 min at 94°C, primer annealing for 1 min at 58°C, and chain extension for 1 min at 72°C; 2 cycles consisting of denaturation for 1 min at 94°C, primer annealing for 1 min at 56°C, and chain extension for 1 min at 72°C; 30 cycles consisting of denaturation for 1 min at 94°C, primer annealing for 1 min at 54°C, and chain extension for 1 min at 72°C; and a final elongation step consisting of 10 min at 72°C. PCR products were analyzed on 1.5% agarose gels and stained with ethidium bromide.

RESULTS

Whole-organism protein electrophoresis. The whole-organism protein patterns of *C. hyoilei* strains were compared with those of type or reference strains representing all species of the genera *Arcobacter*, *Campylobacter*, *Helicobacter*, and *Wolinella*. Figure 2 illustrates the numerical comparison of the whole-cell protein patterns of the type strains or well-established reference strains of all *Campylobacter* species, including three *C. hyoilei* strains and reference strains of *Arcobacter* and *Helicobacter* species. In the resulting dendrogram which was based on a comparison of the complete protein patterns, the *C. hyoilei* strains clustered near the type strain of *C. coli*.

A large number of numerical analyses were subsequently performed; in these analyses we included different sets of reference strains and field isolates present in the database, as described previously (47) (data not shown). *C. coli* and *C. hyoilei* strains always clustered closely together.

Phenotypic analysis. Close agreement between our results and the results described previously for *C. hyoilei* (1) was noted, with two important exceptions. First, we did not find that strain CCUG 33770A produced hydrogen sulfide in triple sugar iron (TSI) agar, even when the period of incubation was extended to 4 days (Table 2). Furthermore, none of the *C. hyoilei* strains that we examined demonstrated the ability to

FIG. 2. Dendrogram derived from the numerical comparison of whole-cell protein patterns of *C. hyoilei* strains and *Campylobacter*, *Arcobacter*, and *Helicobacter* reference strains.

reduce nitrite, although all grew profusely in the test medium. The results described above were consistent when experiments were repeated.

In addition to the interstrain variation in the H₂S-TSI reaction described above, *C. hyoilei* strains differed in the ability to produce DNase and in the ability to grow on crystal violet- and Janus green-containing media (CCUG 33770A gave positive results in the latter three tests). Furthermore, microscopic examination of Gram-stained bacteria revealed characteristic S forms only in slides of CCUG 33450^T and CCUG 33769A preparations, although all cultures contained cells defined as vibrioid (small curved rods). In the remaining 58 tests from the identification scheme of On et al. (27) used, *C. hyoilei* strains proved to be homogeneous and gave results characteristic of *C. coli* in all but three tests (alpha-hemolysis and growth on basic fuchsin- and 5-fluorouracil-containing media). The reproducibility and discriminatory potential of these tests (in addition to differential features defined by Alderton et al. [1]) were thus examined by using type and reference isolates. The data in Table 2 show that unequivocal differentiation between *C. hyoilei* and *C. coli* was not achieved.

DNA-DNA hybridization experiments. DNA-DNA hybridization experiments were performed with the type strains of *C. coli*, *C. hyoilei*, and *C. jejuni*. The DNA-DNA binding level between the *C. hyoilei* and *C. coli* type strains was 67%, while the DNA-DNA binding level measured between the *C. hyoilei* and *C. jejuni* type strains was 35% (the standard errors for these values were about 5%).

GTPase gene assay. DNAs from *C. hyoilei* CCUG 33450^T, CCUG 33769A, and CCUG 33770A were analyzed by amplification of the GTPase gene fragment and Southern blotting. The hybridization pattern typical of *C. coli* strains was obtained for all three isolates, and no cross-hybridization was detected with probes specific for *C. jejuni* or other species. In addition, the sequence of the 156-bp PCR fragment obtained from *C. hyoilei* LMG 15882^T was identical to sequences obtained from multiple *C. coli* reference strains (51).

***glyA* gene assays.** In both *glyA* gene assays, *C. hyoilei* CCUG 33450^T was detected with the same specificity as the *C. coli* type strain (Fig. 1). In the two separate Southern blot hybridizations, the *C. coli*-specific oligonucleotide and *C. coli* CCUG 11283^T *glyA* fragment probes hybridized strongly to the PCR-amplified *glyA* fragment of *C. hyoilei* CCUG 33450^T (Fig. 1).

Both probes detected *C. hyoilei* CCUG 33450^T and *C. coli* CCUG 11283^T with equal intensities (Fig. 1). The *C. jejuni*- and *C. lari*-specific oligonucleotide and *glyA* fragment probes did not hybridize to *C. hyoilei* CCUG 33450^T (data not shown).

***C. jejuni*-*C. coli* multiplex PCR assay.** The three *C. hyoilei* strains and strain LMG 9799 were identified as *C. coli* strains. In the multiplex PCR assay, *C. coli* strains typically produce a 364-bp amplification product, while a 773-bp amplification product is detected with *C. jejuni* strains (50).

DISCUSSION

In the present study, we reexamined the taxonomic affiliation of *C. hyoilei* by using a variety of phenotypic and genotypic criteria. Each analysis clearly indicated that *C. hyoilei* should not be considered a distinct species, and we propose that strains of *C. hyoilei* should be regarded as *C. coli* strains.

Identification of campylobacters by whole-organism protein electrophoresis. It is well-established that numerical analysis of whole-organism protein electrophoresis gels is an excellent tool for species level identification of campylobacters and related bacteria (7–9, 13, 22, 28–30, 32, 42, 43, 45, 47–49, 52). As in many other bacteria (6), a congruence was demonstrated between protein pattern similarity and level of DNA-DNA hybridization, which is the standard for species delineation (21, 32, 42, 45, 49); strains with highly similar whole-cell protein patterns belong to the same species. The presence of a variable dense-band region in *Campylobacter* species often divides species into different protein electrophoretic types, and this region is regularly omitted in numerical analyses to obtain clusters of highly related strains (i.e., strains exhibiting high levels of DNA-DNA binding). This has been illustrated in detail for *Campylobacter* species important in veterinary medicine, including *C. coli* (45). In practice, strains are routinely identified by performing several numerical analyses and changing the number of strains and the region included in the numerical comparison and by visually comparing the protein profiles themselves.

When three *C. hyoilei* strains which were deposited in international culture collections were examined, most analyses revealed that these strains fell into the *C. coli* cluster or into one of the *C. coli* clusters (depending on the number of strains included and their variable regions), suggesting that these strains did not represent a new species but belonged to *C. coli*.

Phenotypic analysis. The close phenotypic resemblance of *C. hyoilei* to *C. coli* was noted in the original description of the former species (1). Nonetheless, Alderton et al. suggested that *C. hyoilei* could be differentiated from *C. coli* by the ability of the former to produce hydrogen sulfide in TSI medium and by the ability to reduce nitrite (1). However, strains of *C. coli* giving positive reactions in the H₂S-TSI (3, 27; this study) and nitrite reduction (3) tests have been noted previously. In addition, the reproducibility of the nitrite reduction test is questionable since we failed to observe this trait in any of the *C. hyoilei* strains studied. The influence of age of the test medium on the outcome of the H₂S-TSI test for *C. coli* is also known (3) and may account for the observation that approximately 40% of *C. coli* preparations yield irreproducible results in this test (as cited by On et al. [27]). We used only freshly prepared TSI medium (at most 3 days old) in this study, and two of three *C. hyoilei* strains and two of six *C. coli* strains gave positive results. These data agree with previously published data for *C. coli* (27).

There are considerable problems associated with comparing campylobacterial phenotypic data obtained by different methods (20). The use of standardized, well-described tests has

been recommended for both identification (20) and description (37) of campylobacterial taxa. The reproducibility of such tests is also clearly important (24, 25), but is often overlooked. We examined *C. hyoilei* with an extensive set of standardized phenotypic tests which have been used to characterize all known *Campylobacter* species and subspecies (27). Although three tests demonstrated some potential to discriminate between *C. hyoilei* and *C. coli*, one test (tolerance to basic fuchsin) was unreliable, and the remaining tests (alpha-hemolysis and tolerance to 5-fluorouracil) proved inadequate, especially when the results were compared to the results obtained with *C. coli* isolates whose identities had been determined by quantitative DNA-DNA hybridization (Table 2).

DNA-DNA hybridization studies. The level of DNA-DNA binding between the *C. hyoilei* and *C. coli* type strains was reported to be 36%, and the DNA base ratio of the former strain was 35 ± 1.5 mol% (1). In order to verify this hybridization value in relation to our data, we hybridized DNA of the *C. hyoilei* type strain with DNAs of the type strains of *C. coli* and *C. jejuni*. We found levels of DNA-DNA binding of 67% between the *C. hyoilei* and *C. coli* type strains and 35% between the *C. hyoilei* and *C. jejuni* type strains. The initial renaturation rate method has been used to study DNA-DNA hybridization levels within and between several *Campylobacter* and *Arcobacter* species, including *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter nitrofigilis*, *Arcobacter skirrowii*, *C. coli*, *Campylobacter concisus*, *C. fetus*, *C. hyointestinalis*, *Campylobacter mucosalis*, and *Campylobacter sputorum* (21, 32, 39, 42, 45, 49). The levels of DNA-DNA hybridization within these species were generally greater than 65%; the only exceptions were *A. cryaerophilus* and *C. concisus* (21, 32, 39, 42, 45, 49). The latter two species, however, are known to comprise multiple genotypic (and phenotypic) subgroups (42, 49). The levels of DNA-DNA hybridization between different species were generally less than 25% (21, 32, 39, 42, 45, 49); the only exceptions were *C. hyointestinalis* and *C. fetus* (significant levels of hybridization between these species were also reported by other workers [46]). We concluded that these results confirm the protein electrophoretic data and indicate that strain CCUG 33450^T and the taxon which it represents belong to *C. coli*.

Our DNA-DNA hybridization results are thus at variance with those of Alderton et al. (1). The problems encountered when DNA-DNA hybridization results obtained by different methods are compared have been discussed in detail previously (46). We used the initial renaturation rate method developed by De Ley and coworkers (10) to study inter- and intraspecific relationships of several *Campylobacter* species and obtained consistent results which correlated with protein electrophoretic results and a variety of other phenotypic markers. This integrated genotypic and phenotypic approach has been successfully used to study relationships of numerous gram-positive and gram-negative bacteria (46).

The reasons for the discrepancy between our results and the results of Alderton et al. (1) are not clear. The methods used by Alderton et al. included a dot hybridization technique which was originally described as a semiquantitative method (16). Some of the strains used in their study were mislabeled, while no strain numbers were provided for several others. A possible explanation for the low level of DNA-DNA binding between the *C. coli* and *C. hyoilei* type strains in the study of Alderton et al. (1) is that the conditions for hybridization were too stringent. It is not contradictory that the remaining strains classified as *C. hyoilei* all exhibited high levels of DNA-DNA binding with the type strain, as these strains were all isolated from pigs with porcine proliferative enteritis and therefore

could represent a particular adapted sublineage of *C. coli*. Indeed, certain strains of *C. hyointestinalis* isolated from porcine gastric mucosae have been shown to be especially highly related based on their DNA-DNA homologies (21), and similar observations have been reported in other genera (53).

16S rRNA sequence analysis. The following levels of 16S rRNA similarity were reported previously (1): 99.7% for the *C. hyoilei* and *C. jejuni* subsp. *doylei* type strains; 99.6% for the *C. hyoilei* and *C. jejuni* subsp. *jejuni* type strains; and 98.5% for the *C. hyoilei* and *C. coli* type strains. It has been documented that the resolution of 16S rRNA sequence analysis for closely related species is low and that for species level identification comparisons of similarity levels greater than 97% are not appropriate (11, 35). For instance, a representative urease-positive thermophilic campylobacter strain (CCUG 18267 = LMG 7791 = NCTC 11845), which has been reported by Alderton et al. to share 99.2% of its 16S rRNA sequence with the type strains of *C. jejuni* subsp. *doylei*, *C. jejuni* subsp. *jejuni*, and *C. lari*, exhibits a significant DNA-DNA binding level (67%) with the type strain of *C. lari* (39), but not with the type strains of the *C. jejuni* subspecies. Protein electrophoretic analysis of this strain clearly identifies it as *C. lari* (29, 47), as does a numerical analysis of phenotypic test results (26). A similar situation occurred in the present study; the higher level of 16S rRNA similarity between the *C. hyoilei* and *C. jejuni* type strains compared to the level of similarity between the 16S rRNA sequences of the *C. hyoilei* and the *C. coli* type strains did not correlate with overall DNA homology as expressed by DNA-DNA hybridization values.

PCR and probe-based identification assays. The technological burst in molecular biology has resulted in several genome-oriented identification methods for campylobacters (see reference 20 for a review). Alderton et al. (1) reported that *C. hyoilei* strains were indistinguishable from *C. coli* strains in their DNA probe hybridization assays. In Southern blots obtained by using a 6.1-kb insert from the *C. jejuni* genome as the probe and genomic DNA digested with three different restriction enzymes, identical patterns were always detected for *C. hyoilei* and *C. coli* strains. In our analysis, we included *C. hyoilei* strains in several identification protocols based on known or novel genes or on randomly generated DNA fragments. In each of the different approaches, *C. hyoilei* strains always reacted like typical *C. coli* strains.

Conclusion. DNA reassociation studies currently represent the "gold standard" for species delineation (35, 54). Strains that exhibit approximately 70% or greater DNA-DNA relatedness should be assigned to the same species (54). Furthermore, it has been recommended that genomically distinct taxa should not be formally named unless they can be identified by some phenotypic property (38, 54). On the basis of both of these fundamental criteria, we submit that the taxonomic standing of *C. hyoilei* as a distinct species cannot be justified. All of our other data clearly support this view. It is conceivable that the *C. hyoilei* strains represent a variant of *C. coli* that is highly adapted for the porcine enteric tract with pathological consequences for the animal. Thus, we suggest that the epithet of *C. hyoilei* could be retained as an infraspecific designation. We recommend that further studies be undertaken to clarify whether an infraspecific designation can be validated by virtue of differential biochemical and/or pathological behavior in such strains. Until such data are available, we propose that *C. hyoilei* Alderton et al. 1995 should be regarded as a junior synonym of *C. coli* Véron and Chatelain 1973.

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