Helicobacter salomonis sp. nov., a Canine Gastric *Helicobacter* sp. Related to *Helicobacter felis* and *Helicobacter bizzozeronii*

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During a study of the prevalence and distribution of gastric helicobacters in domestic pets, a novel group of *Helicobacter*-like organisms were identified. These “*Helicobacter* group 2” strains were initially distinguished from the species *Helicobacter felis* and *Helicobacter bizzozeronii* by their cellular morphology and the type of motility exhibited. Bacterial cells were only slightly spiral, 5 to 7 µm long, and 0.8 to 1.2 µm wide and showed an unusual slow wavelike motion. Each cell had tufts of sheathed flagella at one or both ends. Phylogenetic analysis by 16S ribosomal DNA sequence comparison revealed that *H. felis*, *H. bizzozeronii*, “*Gastrospirillum hominis*” 2, and the new group of helicobacters formed a distinct cluster with intraspecies similarity values of more than 98%. These taxa were clearly separated from all other known *Helicobacter* species. Dot blot DNA-DNA hybridization studies indicated that the *Helicobacter* group 2 strains are genetically homogeneous and distinct from other canine and feline gastric helicobacters. Quantitative DNA-DNA hybridization experiments showed that *Helicobacter* group 2 strains exhibit >90% DNA homology to each other, but <39% homology to the phylogenetically related taxa *H. felis* and *H. bizzozeronii*. We propose the name *Helicobacter salomonis* for the novel *Helicobacter* group 2 strains. The type strain is *H. salomonis* Inkinen (= CCUG 37845).

Since the end of the 19th century, spiral organisms have been observed in the gastric mucosa of both dogs and cats (29, 31). In 1970, Lockard and Boler (22) suggested that three morphologically distinct bacteria could be observed in the canine gastric mucosa, although these bacteria were believed to represent different stages of movement of the same organism (22). Further study of these bacteria was impaired by the lack of suitable methods of culture. However, interest in gastric spiral bacteria increased with the isolation of *Helicobacter pylori* (36) and the consequent realization of its clinical and economic importance. This interest, combined with the need for a suitable animal model for *H. pylori* infection, led to intense research concerning the presence of similar bacteria in other animal species. As a consequence, there has been a rapid expansion of the number of *Helicobacter* spp. described. At present, 16 *Helicobacter* species isolated from humans and different animals have been named (7, 23, 24).

The first canine gastric *Helicobacter* sp. isolated in vitro was *Helicobacter felis*, a large, tightly spiral organism with fibrils wrapped around its cell body (21). A similar organism that lacks periplasmic fibrils is also known to be common in dogs and cats (12, 14, 17, 21). Recently, a group of organisms showing this morphology has been cultured from dogs and named *Helicobacter bizzozeronii* (12). Spiral bacteria ultrastructurally similar to the latter species have been observed in human, pig, and lemur gastric mucosa and provisionally assigned to the genus “*Gastrospirillum*” (4, 16, 28, 32). However, the name “*Helicobacter heilmannii*” has been proposed for the human organism, although this name does not distinguish from the two phylogenetically divergent variants that have been identified (32). A culture of a human isolate resembling “*H. heilmannii*” has been described recently (1). Another organism, which

is more cylindrical and has numerous filaments around the cell body, has also been seen in canine gastric mucosa (22). This organism resembles “*Flexispira rappini*,” a *Helicobacter*-like organism isolated from aborted ovine fetuses (20) and human diarrheal samples (2).

On the basis of DNA-rRNA hybridization data, the genus *Helicobacter* belongs to rRNA homology group III of rRNA superfamily VI (35). 16S rRNA sequence comparisons have shown that “*F. rappini*” and “*Gastrospirillum*” strains are closely related to *Helicobacter* species (26), with the gastrospirilla forming a discrete phylogenetic cluster within the genus (32).

During a study of the prevalence and species distribution of canine gastric helicobacters, we isolated strains (initially referred to as “*Helicobacter* group 2”) that differed in morphology and in motility characteristics from *H. felis* and *H. bizzozeronii*. We present here phenotypic, genotypic, and phylogenetic data showing that *Helicobacter* group 2 is a new species which is closely related to other canine gastric helicobacters. The name *Helicobacter salomonis* is proposed for this taxon.

MATERIALS AND METHODS

Isolation. Helicobacters were isolated from biopsy samples from healthy pet dogs and adult experimental beagle dogs at the Faculty of Veterinary Medicine. Biopsy, bacterial culture, and primary identification procedures were as described previously (11, 12). All of the spiral bacteria isolated were stored in sterile skim milk supplemented with 20% glycerol at –70°C. The *Helicobacter* group 2 strains and other type and reference strains used in this study are shown in Table 1.

Gross morphology and motility. Giemsa staining was used to detect the organisms from gastric mucosal brush cytology samples as described previously (13). Gram staining was used to visualize the morphology of the cultured organisms. To confirm that the organism isolated belonged to putative *Helicobacter* group 2, a drop of culture was placed onto a slide and covered with a coverslip, and motility was observed by using an oil immersion method and a light microscope.

Transmission electron microscopy. Bacterial cells were resuspended in physiological NaCl, and a turbid suspension was stained with 1% phosphotungstic acid and examined with a JEOL model 1200 EX electron microscope, as described previously (12).

Biochemical and tolerance tests. Commonly used phenotypic tests recommended by Ursing et al. (34) for the description of new *Campylobacter* species, by Goodwin and Armstrong (9) for studies of *H. pylori*, and by Hänninen et al. (12) for studies of *H. bizzozeronii* were used for characterization. The strains were

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TABLE 1. Bacterial strains

Taxon	Strain(s) ^a	Sender ^b	Source	Nucleotide sequence accession no.
<i>H. salomonis</i> isolate 1	Elviira I		Canine gastric mucosa	
<i>H. salomonis</i> isolate 2	Elviira II		Canine gastric mucosa	
<i>H. salomonis</i> isolate 3	Alma		Canine gastric mucosa	
<i>H. salomonis</i> isolate 4	06A (= CCUG 37848)		Canine gastric mucosa	Y09405
<i>H. salomonis</i>	Vilho		Canine gastric mucosa	
<i>H. salomonis</i>	Mini		Canine gastric mucosa	
<i>H. salomonis</i>	Inkinen ^T (= CCUG 37845 ^T)		Canine gastric mucosa	U89351
<i>H. salomonis</i>	Ko.K. III		Canine gastric mucosa	
" <i>F. rappini</i> "	ATCC 43966 ^c		Aborted sheep fetus and liver	M88137
" <i>F. rappini</i> "	Hilli		Canine gastric mucosa	
" <i>G. hominis</i> " 1	Unculturable ^c		Human gastric mucosa	L10079
" <i>G. hominis</i> " 2	Unculturable ^c		Human gastric mucosa	L10080
<i>H. acinonyx</i>	CCUG 29263 ^T (= ATCC 51101 ^T)	CCUG	Cheetah gastric mucosa	M88148
<i>H. bilis</i>	ATCC 51630 ^{Tc}		Murine liver	U18766
<i>H. bizzozeronii</i>	Storkis ^T (= CCUG 35545 ^T)		Canine gastric mucosa	Y09404
<i>H. bizzozeronii</i>	14 (= CCUG 35046)		Canine gastric mucosa	
<i>H. bizzozeronii</i>	Wiberg		Canine gastric mucosa	
<i>H. bizzozeronii</i>	5F		Canine gastric mucosa	
<i>H. bizzozeronii</i>	10		Canine gastric mucosa	
<i>H. bizzozeronii</i>	12A		Canine gastric mucosa	
<i>H. canis</i>	CCUG 12739 ^T	CCUG	Canine feces	L13464
<i>H. cinaedi</i>	ATCC 35683 ^{Tc}		Human feces	M88150
<i>H. felis</i>	CS1 ^T (= ATCC 49179 ^T)	O'Rourke	Feline gastric mucosa	M57398
<i>H. felis</i>	DS3	O'Rourke	Canine gastric mucosa	M37643
<i>H. felis</i>	DS4 (= CCUG 28540)	CCUG	Canine gastric mucosa	
<i>H. felis</i>	1602 (= Dog 1) ^c	Eaton	Canine gastric mucosa	U51870
<i>H. felis</i>	2301 (= Dog 2 = CCUG 37850) ^c	Eaton	Canine gastric mucosa	U51871
<i>H. felis</i>	Dog 7, (= Dog 3)	Eaton	Canine gastric mucosa	U51872
<i>H. fennelliae</i>	ATCC 35684 ^{Tc}		Human feces	M88154
<i>H. hepaticus</i>	ATCC 51448 ^{Tc}		Murine liver	U07574
<i>H. mustelae</i>	ATCC 43772 ^T	ATCC	Ferret gastric mucosa	M35048
<i>H. muridarum</i>	ATCC 49282 ^{Tc}		Murine intestinal mucosa	M80205
<i>H. nemestrinae</i>	ATCC 49396 ^{Tc}		Macaque gastric mucosa	X67854
<i>H. pametensis</i>	CCUG 29255 ^{Tc}		Tern feces	M88147
<i>H. pullorum</i>	NCTC 12824 ^{Tc}		Broiler chicken cecum	L36141
<i>H. pylori</i>	Tx30a (= ATCC 51932)	ATCC	Human gastric mucosa	
<i>H. pylori</i>	ATCC 43504 ^{Tc}		Human gastric mucosa	M88157
<i>H. trogonum</i>	ATCC 700114 ^{Tc}		Rat colon mucosa	U65103

^a ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; Eaton, K. A. Eaton, Department of Veterinary Pathobiology, Ohio State University, Columbus; O'Rourke, J. L. O'Rourke, School of Microbiology and Immunology, University of New South Wales, Sydney, Australia.

^b Our isolate unless indicated otherwise.

^c Only 16S rRNA sequence data were used in this study.

first tested for oxidase, catalase (3% hydrogen peroxide), rapid urease, and hippurate hydrolysis reactions. All strains were also tested by using API Campy test strips (Biomérieux SA, Marcy l'Etoile, France) and Rosco diagnostic tablets (Rosco Diagnostica, Taastrup, Denmark). These tests included tests for urease; nitrate reductase; indoxyl acetate hydrolysis; hippurate hydrolysis; triphenyltetrazolium chloride (TTC) reduction; gamma-glutamyl transpeptidase; pyrrolidonyl, L-arginine, and L-aspartate arylamidases; and alkaline phosphatase. DNase production was also tested (10).

The following tolerance tests were done on brucella (Oxoid, Basingstoke, United Kingdom) blood agar containing 1% glycine, 1% ox bile (Difco Laboratories, Detroit, Mich.), and 2.5% NaCl. Antibiotic sensitivity was tested on brucella blood agar supplemented with nalidixic acid (30-µg disks; AB Biodisk, Solna, Sweden), cephalothin (30-µg disks; AB Biodisk), metronidazole (50-µg disks; Rosco), and cefoperazone (60-µg disks; Rosco). All growth and tolerance tests were incubated for up to 7 days in a microaerobic atmosphere at 37°C.

Growth conditions. Growth of the organisms was tested on brain heart infusion (BHI) (Oxoid) blood agar, brucella (Oxoid) blood agar, nutrient (Oxoid) blood agar, and Mueller-Hinton (Oxoid) blood agar. Growth at 25, 30, 37, 40, and 42°C was tested on brucella blood agar. Growth in liquid media was tested by using brucella broth (Oxoid) containing 5% horse serum and 0.1% soluble starch. A shallow medium was used, and during incubation the media were continuously shaken at 100 rpm. The cultures were incubated for 7 days in a microaerobic atmosphere at 37°C.

Amplification of 16S ribosomal DNA by PCR and purification of the PCR product. The 16S rRNA gene sequence of selected canine helicobacter strains (see below for details) was obtained from genomic bacterial DNA by PCR

amplification with two primers, biotinylated primer HY-2B (pGCCTAATACA TGCAAGTCGAACG; anneals to positions 45 to 67 of *Escherichia coli* 16S rRNA) and primer A-2 (pCGGTTACCTTGTACGACTTC; positions 1524 to 1541). The PCR conditions used have been described previously (38), and purification of single-stranded DNA from biotinylated PCR product was performed as described previously (38).

Sequencing. The 16S rRNA gene sequences of *Helicobacter* group 2 strain 06A and *H. bizzozeronii* CCUG 35545^T were determined as follows. Nucleotide sequences were determined by using Sanger's dideoxy method and Sequenase 2.0R polymerase (Amersham, Arlington Heights, Ill.) according to the manufacturer's recommendations. To sequence the partial 16S rRNA gene sequences directly from PCR templates, we used primer A-2 together with the following five additional primers: R1 (pCTGCTGGCAGGAGTTAGCC; positions 505 to 525), R339 (pACTGCTGCCTCCCGTAGGAG; positions 339 to 358), R4 (pCC AGGGTATGTAATCCTCTT; positions 781 to 800), R5 (pGTGACGACAGC CATCAGCA; positions 1049 to 1068), and R6 (pTGTGACGGGCGGTGTG TACA; positions 1391 to 1410). Ten milliliters of avidin-coated Pandex beads (IDEXX, Westbrook, France) with attached single-stranded PCR product was used as the template in the dideoxy sequencing reactions. The 16S rRNA gene sequence of *Helicobacter* group 2 strain Inkinen^T was determined by PCR amplification of the 16S rRNA gene and cycle sequencing as described previously (6).

Phylogenetic analysis. The 16S rRNA gene sequences were entered into RNA, a program for analysis of 16S rRNA data in Microsoft Quickbasic for use with IBM PC-compatible computers, and aligned as previously described (25). The database used contains approximately 100 *Helicobacter*, *Wolinella*, *Arcobacter*,

and *Campylobacter* sequences and more than 900 sequences for other bacteria. Similarity matrices were constructed from aligned sequences by using only those base positions for which 90% of the strains had data and were corrected for multiple base changes by the method of Jukes and Cantor (19). Phylogenetic trees were constructed by the neighbor-joining method (30). The nucleotide sequence accession numbers for the strains examined in this study are listed in Table 1.

DNA isolation. To obtain sufficient quantities of DNA for DNA-DNA hybridizations, a few hundred plates of each strain were cultured under the conditions described previously (11, 12). DNA was isolated by the method of Pitcher et al. (27), with modifications designed to remove the RNA and proteins. RNase and proteinase K treatments were performed after dissolution in TE (10 mM Tris-HCl, 1 mM EDTA), and the DNA was reprecipitated as in the first phase.

Dot blot DNA-DNA hybridizations. Dot blot DNA-DNA hybridizations were performed by dot blot assay on nylon membranes (Micron Separations, Inc., Westborough, Mass.) by using a vacuum-blotting apparatus (Pharmacia LKB Biotechnology, Uppsala, Sweden); 50-, 5-, and 0.5-ng aliquots of diluted chromosomal DNA were added to the nylon membranes. The type and reference strains used were *H. felis* CS1^T, DS3, and DS4, *H. bizzozeronii* CCUG 35545^T, CCUG 35046, Wiberg, 5F, 10, and 12A, *H. pylori* Tx30a, *Helicobacter mustelae* ATCC 43772^T, *Helicobacter acinonyx* CCUG 29263^T, and "*F. rappini*" Hilli (Table 1). The probes used were genomic DNAs from *H. bizzozeronii* CCUG 35545^T, *H. salomonis* Elviira II, and *H. felis* CS1^T and DS3 (Table 1). Hybridizations were performed as described previously (12), except that three different temperatures (58, 68, and 74°C) were used. The tests were repeated three times.

Optical DNA-DNA hybridization. Optical DNA-DNA hybridization was performed essentially by the initial reassociation method of De Ley (3) with a DNA concentration of 35 µg/ml as recommended by Huss et al. (18). The DNA samples were adjusted to a concentration of approximately 300 µg/ml by adding 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) to the initial 0.1× SSC to reach a concentration of approximately 2× SSC. DNA was subsequently fragmented by sonication and dialyzed against 2× SSC overnight at 4°C. The samples were stored at -20°C before measurement. Hybridization was performed with a Lambda 11 spectrophotometer by using a type PTP 6 heating element (Perkin-Elmer, Ueberlingen, Germany) and Winlab and Templab software (Perkin-Elmer). Renaturation of the DNA was performed as recommended previously (15). The optimal reassociation temperature (69°C) was used, as recommended by Gillis et al. (8). The degree of binding was calculated by using the formula recommended by De Ley (3). Hybridizations were performed at least twice except where stated otherwise.

RESULTS

Culture. Five strains were isolated from corpus biopsies of dogs. One of the strains was represented by four isolates. Two of these isolates were from two experimental beagle bitches (bitches A and E) from the same supplier of experimental animals; the other two isolates of this strain were subsequently isolated from the offspring of these bitches. Ribotyping of all of these isolates with three different restriction enzymes suggested that they represented the same bacterial clone (data not shown). The sources and designations of the strains are shown in Table 1. Primary isolation of *Helicobacter* group 2 strains from biopsy material usually occurred after 3 to 6 days of incubation, and the growth appeared as a thin, nonhemolytic spreading film. In subcultures, bacterial growth was more rapid and profuse and was normally observed within 2 days. All strains showed some growth in brucella broth supplemented with horse serum. As the cultures aged, coccal forms were seen.

Gross morphology and motility. In Giemsa-stained preparations of direct mucosal brush cytology samples, *Helicobacter* group 2 cells were shorter and less spiral than *H. bizzozeronii* and *H. felis* cells in comparable samples (Fig. 1). Gram-stained *Helicobacter* group 2 cells were rather thick, and the cell body was only slightly spiral. The motility of the strains as determined by light microscopy was rather slow and wavy. By contrast, the motility of *H. felis* and *H. bizzozeronii* was rapid, and movement was attained by means of a vigorous corkscrew-like action. The motility properties were observed in 48-h cultures.

Ultrastructure. As determined by transmission electron microscopy, *Helicobacter* group 2 cells were loosely helical or wavy rods, 5 to 7 µm long and 0.8 to 1.2 µm wide. They had multiple sheathed flagella at one or both ends, and no fibrils

FIG. 1. Giemsa-stained brush cytology samples from the gastric mucosae of a beagle puppy naturally infected with *H. salomonis* 06A (= CCUG 37848) (A) and a beagle puppy infected intentionally with *H. bizzozeronii* Storkis^T (= CCUG 35545^T) (B). Note the thicker appearance and less helical cell body of *H. salomonis*. Bar = 5 µm.

were seen (Fig. 2). The cell bodies of *Helicobacter* group 2 strains were shorter and thicker than the cell bodies of *H. bizzozeronii* (Fig. 1 and 2 and Table 2). The cell body of *Helicobacter* group 2 was markedly less helical than that of *H. felis* or *H. bizzozeronii*.

Biochemical and tolerance tests. Thirty tests were used to characterize the *Helicobacter* group 2 isolates. All of the strains were oxidase, catalase, and urease positive and grew on blood-supplemented BHI, brucella, nutrient, and Mueller-Hinton agar media. The best growth was obtained on BHI and brucella agar media. The strains also grew weakly in brucella broth supplemented with 0.1% soluble starch and 5% horse serum. *Helicobacter* group 2 phenotypically resembled *H. bizzozeronii* and *H. felis* and had similar antibiotic susceptibilities, although the isolates from two dams and their puppies were resistant to metronidazole. *Helicobacter* group 2 strains could nonetheless be differentiated from both *H. bizzozeronii* and *H. felis* by their ability to hydrolyze indoxyl acetate, by their ability to reduce TCC, and by their inability to grow at temperatures higher than 40°C. The salient tests that distinguish *Helicobacter* group 2 from other canine gastric helicobacters are listed in Table 2.

Sequencing and phylogenetic analysis. Approximately 95% of the 16S rRNA sequence was determined for each of the

FIG. 2. Transmission electron micrograph of *H. salomonis*. Note the multiple sheathed flagella and the slight loss of spiral morphology compared to Fig. 1A. Bar = 1 μ m.

Helicobacter strains examined. A similarity matrix based on comparisons of 16S rRNA sequences of 24 strains representing 19 *Helicobacter* species is shown in Table 3. Only those base positions (1,404 positions) for which 90% of the strains possessed data were included in the similarity calculation. A phylogenetic tree based on this analysis is shown in Fig. 3. This analysis showed that the novel *Helicobacter* group 2 strains, *H. felis*, *H. bizzozeronii*, and "*Gastrospirillum hominis*" 2 were

highly related and formed a distinct cluster within rRNA homology group III (i.e., the *Helicobacter* phylogenetic branch) of rRNA superfamily VI.

Dot blot DNA-DNA hybridization. Dot blot DNA-DNA hybridization at 74°C revealed that the *Helicobacter* group 2 probe did not hybridize intensively either with *H. felis* DNA or with *H. bizzozeronii* DNA, but hybridized strongly with DNAs from all of the other *Helicobacter* group 2 isolates. Similarly, the *H. bizzozeronii* CCUG 35545^T probe hybridized with all strains of *H. bizzozeronii*. Both *H. felis* CS1^T- and DS3-derived probes hybridized with all *H. felis* strains. The results obtained in experiments performed at 68 and 74°C were the same; 58°C proved to be less stringent.

Optical DNA-DNA hybridization. The *Helicobacter* group 2 strains selected for the optical DNA-DNA hybridization experiments gave interstrain DNA reassociation values of more than 90% (Table 4). The levels of DNA-DNA hybridization between *Helicobacter* group 2 strains and strains of *H. felis* and *H. bizzozeronii* were significantly lower (11 to 39%) (Table 4).

DISCUSSION

It has been known for a century that the gastric mucosa of dogs, cats, and other species may harbor up to three morphologically different spiral organisms (10), the clinical significance of which has remained unclear (5, 14). These bacteria have long been considered to be unculturable (21), and their characterization has been mainly limited to ultrastructural studies. More recently, sequence analysis of the 16S rRNA gene has been used (32). The general lack of information about these bacteria has resulted in confusion in their classification and nomenclature. Older literature treats these organisms as spirochetes and spirilla among other things, while using 16S rRNA sequence data to classify these bacteria has resulted in

TABLE 2. Characteristics of *H. salomonis* and related gastric helicobacters^a

Characteristic	<i>H. salomonis</i>	<i>H. bizzozeronii</i>	<i>H. felis</i>	<i>H. pylori</i>	<i>H. acinonyx</i>	" <i>F. rappini</i> "
Cell length (μ m)	5-7	5-10	5-7.5	2.5-5	1.5-2.0	6-15
Cell width (μ m)	0.8-1.2	0.3	0.4	0.5-1.0	0.3	0.5-1.0
Periplasmic fibrils	—	—	+	—	+	+
Location of flagella	Bipolar	Bipolar	Bipolar	Polar	Polar	Bipolar
No. of flagella	10-23	10-20	14-20	4-8	2-5	10-20
Flagellar sheath	+	+	+	+	+	+
Catalase activity	+(5/5) ^b	+	+	+	+	+
Oxidase activity	+(5/5)	+	+	+	+	+
Urease activity (rapid)	+(5/5)	+	+	+	+	+
Nitrate reduction	+(5/5)	+	+	—	—	—
Hippurate hydrolysis	—(0/5)	—	—	—	—	—
Indoxyl acetate hydrolysis	+(5/5)	+	—	—	—	+
γ -Glutamyl aminopeptidase	+(5/5)	+	+	+	+	+
TTC reduction	+(5/5)	+	—	+	ND	+
Alkaline phosphatase activity	+(5/5)	+	+	+	+	+
DNase activity	+(4/5)	+	+	+	ND	ND
Growth at:						
25°C	—(0/5)	—	—	—	—	—
37°C	+(5/5)	+	+	+	+	+
40°C	(+)(3/3)	ND	ND	ND	ND	ND
42°C	—(0/5)	+	—	—	+	—
Tolerance to 1% ox bile	—(0/5)	—	—	—	—	—
Susceptibility to:						
Nalidixic acid (30- μ g disk)	R (0/5)	R	R	R	R	S
Cephalothin (30- μ g disk)	S (5/5)	S	S	S	R	S
Cefoperazone (60- μ g disk)	S (5/5)	S	S	ND	ND	S
Metronidazole (50- μ g disk)	S (4/5)	S	S	S	S	S

^a —, negative or not present; +, positive or present; ND, not determined; R, resistant; S, susceptible.

^b The data in parentheses are the number of strains that have the characteristic indicated/number of strains tested.

TABLE 3. Similarity matrix based on 16S rRNA sequence comparisons

Taxon	% Similarity or % difference ^a																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
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^a The numbers on the upper right are uncorrected percentages of similarity, and the numbers on the lower left are percentages of difference corrected for multiple base changes by the method of Jukes and Cantor (19).

FIG. 3. Phylogenetic tree for 20 strains of *Helicobacter* species based on 16S rRNA sequence similarity. The scale bar represents a 4% difference in nucleotide sequences as determined by measuring the lengths of the horizontal lines connecting any two species.

different taxonomic problems. For example, sequences purported to represent two distinct species infecting humans ("*G. hominis*" 1 and "*G. hominis*" 2) have both been called "*H. heilmannii*" (32). The only validly described species showing a distinctive spiral ultrastructure similar to the gastrospirilla are *H. felis* (26) and *H. bizzozeronii* (12).

In direct Giemsa-stained preparations of brush cytology samples, *Helicobacter* group 2 strains were observed to be coiled bacilli. In culture, this morphology altered, and only loosely helical or slightly curved thick forms were seen in Gram-stained bacterial films, in contrast to comparable preparations of *H. felis* and *H. bizzozeronii*, which retained a distinct spiral morphology. In addition, the motility of *Helicobacter* group 2 strains was wavelike and slow compared with both *H. felis* and *H. bizzozeronii*. This might be due to the less pronounced spiral cell body of *Helicobacter* group 2; we noted that *H. felis* and *H. bizzozeronii* were able to move in a rapid, screwlike fashion. In electron micrographs, *Helicobacter* group 2 isolates were less helical and thicker than *H. bizzozeronii* and did not have the periplasmic fibrils that are seen typically in *H. felis*. Indeed, Weber and Schmittiel (37) may have observed *Helicobacter* group 2 strains in their investigations of gastric spiral bacteria in dogs, since the organism displayed in Fig. 4 of their paper has a marked resemblance to the organisms which we describe here.

The cultural characteristics of *H. felis*, *H. bizzozeronii*, and *Helicobacter* group 2 were similar. After primary isolation, *H. felis* and *Helicobacter* group 2 showed profuse growth within 2 days, while *H. bizzozeronii* needed 3 to 4 days for visible growth to appear. *H. bizzozeronii* was the only one of these taxa to show some growth at 42°C. *Helicobacter* group 2 showed growth in broth media. Conversely, *H. bizzozeronii* did not show any turbidity in broth cultures (12). Both *H. bizzozeronii*

and *Helicobacter* group 2 were able to hydrolyze indoxyl acetate and reduce TTC, unlike *H. felis*. All canine gastric helicobacter cells exhibit coccal transformation as the cultures age.

It should be noted that the gastric mucosa of dogs may be colonized by several *Helicobacter* species simultaneously. Since the isolation procedures for and cultural characteristics of all canine gastric helicobacters are very similar (12, 21), mixed cultures of different *Helicobacter* species or different strains of the same species may be obtained. All of our strains were isolated during 1994 and 1995. They were all subcultured at least 10 to 20 times. To ascertain the purity of the strains, motility tests and Gram staining were performed after almost every subculture. Visual examination of sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein profiles and ribotyping with three different enzymes were performed on several occasions (data not shown). All of the strains described in this paper remained similar throughout this study. We obtained one known mixed culture of *H. salomonis* and *H. bizzozeronii* which was omitted from this study.

Recently, on the basis of high 16S rRNA sequence similarity values, Eaton et al. (5) described three *H. felis* organisms without periplasmic fibrils. Their strain Dog 3 exhibited sequence differences of 0.7 and 0.6% with *H. felis* CS1^T and DS3, respectively. Our quantitative DNA-DNA hybridization study performed with strain Dog 3 revealed a level of DNA relatedness of 66% with *H. felis* CS1^T. This strain hybridized 12 and 26% with *H. bizzozeronii* CCUG 35545^T and *Helicobacter* group 2 strain Inkien^T, respectively.

The only cultured isolate of the human gastrospirillum group ("*H. heilmannii*") has been reported to form small, uniform, translucent colonies similar to those of *H. pylori* in primary isolation preparations after 5 days of incubation (1). None of our canine gastric helicobacters has shown this type of growth. However, a more detailed polyphasic study is necessary to ascertain the relationship of this human isolate to *Helicobacter* group 2 (described here), *H. bizzozeronii*, *H. felis*, "*G. hominis*" 1, "*G. hominis*" 2, "*Gastrospirillum suis*," and "*Gastrospirillum lemur*."

The 16S rRNA sequences of strains representing *H. felis*, *H. bizzozeronii*, and *Helicobacter* group 2 were 98.2 to 100% similar to one another, clearly indicating a close phylogenetic relationship. *Helicobacter* group 2 strain Inkien^T differed from *H. bizzozeronii*, *H. felis*, and "*G. hominis*" 2 by 1.0, 0.8, and 1.4%, respectively. Furthermore, the two sequences *Helicobacter* group 2 strains differed by 1.7% from each other. Because of the high degrees of sequence similarity among these *Helicobacter* species and sequence variability within the species,

TABLE 4. Optical DNA-DNA reassociation values

Strain	% Optical DNA-DNA reassociation with:		
	<i>H. salomonis</i> Inkien ^T	<i>H. bizzozeronii</i> Storkis ^T	<i>H. felis</i> CS1 ^T
<i>H. salomonis</i> Elviira II	100	34	34
<i>H. salomonis</i> Mini	92	34 ^a	39 ^a
<i>H. salomonis</i> Inkien ^T (= CCUG 37845 ^T)	100	11 ^a	38 ^a
<i>H. bizzozeronii</i> Storkis ^T (= CCUG 35545 ^T)	11 ^a	100	22
<i>H. bizzozeronii</i> 14 (= CCUG 35046)	23	89	2
<i>H. bizzozeronii</i> 12A	33	74	20
<i>H. felis</i> CS1 ^T (= ATCC 49179 ^T)	38	22	100
<i>H. felis</i> DS3	37 ^a	30 ^a	79 ^a
<i>H. felis</i> Dog 3	26	12	66 ^a

^a Only one result was obtained.

16S rRNA sequence information cannot be used to establish species boundaries (33). The accepted gold standard of DNA-DNA hybridization was therefore used to delimit species boundaries for the strains in this cluster.

The dot blot DNA-DNA hybridization method was found to be a good screening method which could be used to select strains for the quantitative DNA-DNA reassociation study. Representatives of *H. felis*, *H. bizzozeronii*, and *Helicobacter* group 2 were confirmed by using the quantitative DNA-DNA reassociation method to identify separate species. Therefore, as determined by a variety of phenotypic and genotypic criteria, *Helicobacter* group 2 can be shown to be a distinct, novel *Helicobacter* species, for which we propose the name *H. salomonis*.

Description of *Helicobacter salomonis* sp. nov. *Helicobacter salomonis* (sa.lo.mo'nis, L. gen. n. *salomonis*, of Salomon; in honor of Hugo Salomon, a German scientist who was one of the first workers to describe three morphologically different spiral organisms in canine gastric mucosa). The cells in vitro cultures are loose spirals that are 0.8 to 1.2 μ m wide by 5 to 7 μ m long. They do not have periplasmic fibrils. In older cultures coccoids predominate. The cells are gram negative and non-sporulating. They are motile by means of tufts of 10 to 23 sheathed flagella at one or both ends of the cell; the movement is slower than that of *H. felis* or *H. bizzozeronii*. Individual colonies are not formed, but cultures grow as spreading films on fresh moist agar media. All strains are oxidase, catalase, and urease positive. They reduce nitrate and TTC, and they are positive in indoxyl acetate, gamma-glutamyl transpeptidase, and alkaline phosphatase tests. They are negative in hippurate hydrolysis, pyrrolidonyl arylamidase, L-arginine arylamidase, and L-aspartate arylamidase tests. Four of the five strains produce DNase. They are resistant to nalidixic acid and are susceptible to cephalothin and cefoperazone. One strain shows resistance to metronidazole. They do not grow on media containing 1% ox bile, 1% glycine, or 1.5% NaCl. They grow at 37°C, but not at 25 or 42°C. All of the biochemical and tolerance characteristics are similar to the characteristics of *H. bizzozeronii*, except that one strain of *H. salomonis* is DNase negative and one strain is resistant to metronidazole. The clinical significance of *H. salomonis* is unknown.

Description of the type strain. Strain CCUG 37845 (= Inkinen) is the type strain of *H. salomonis*. This strain conforms to the description given above. It was isolated from a gastric biopsy of a healthy pet dog. An additional strain, strain 06A, has been deposited in the Culture Collection of the University of Göteborg as strain CCUG 37848.

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