High-Resolution Genotyping of *Campylobacter upsaliensis* Strains Originating from Three Continents

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Ninety-six *Campylobacter upsaliensis* strains that originated from Australia, Canada, and Europe (Germany) and that were isolated from humans, dogs, and cats were serotyped for their heat-stable surface antigens. All of them were genotyped by enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR) profiling, and 83 strains were genotyped by macrorestriction analysis with the endonuclease XhoI. Eighty-four percent of the strains belonged to five different serotypes (serotypes OI, OII, OIII, OIV, and OVI), with the proportions of strains in each serotype being comparable among the groups of strains from all three continents. Two serotypes, OIII and OIV, were prevalent at rates of 35 to 40%. Serotypes OI, OII, and OVI were detected at rates of 1.5 to 15%. Between 10 and 17.7% of the strains did not react with the available antisera. Analysis of the ERIC-PCR profiles revealed two distinct genotypic clusters, which represented the German and the non-European strains, respectively. XhoI macrorestriction yielded two genotypic clusters; one of them contained 80.2% of the German strains and 34.6% of the non-European strains, and the second cluster consisted of 65.4% of the non-European strains and 19.8% of the German strains. Fourteen strains from all three continents were analyzed for their 16S rRNA gene sequences. Only two minor variations were detected in four of the strains. In conclusion, *C. upsaliensis* has undergone diverging processes of genome arrangement on different continents during evolution without segregating into different subspecies.

Campylobacter upsaliensis, a catalase-negative or weakly catalase positive thermotolerant *Campylobacter* species, was first isolated in 1983 from fecal samples of healthy and diarrheic dogs in Sweden (53). A few years later, in 1989, this microorganism was identified in cats (10). Several reports concerning the carriage rates in dogs and cats and the risk that humans can acquire infections from pet animals have been published (2, 5, 6, 15, 18, 33, 34, 39). Cases of clinical disease in humans caused by infections with *C. upsaliensis* have been reported from different areas of the world. *C. upsaliensis* was isolated from patients with abortion (17), bacteremia (19, 29, 44), an abscess (11), gastroenteritis (12, 13, 14, 26, 29, 30, 44, 48, 56; W. M. Johnson, D. L. Woodward, R. Khakhria, and L. J. Price, *Campylobacter, Helicobacter* and Related Organisms, Proc. 9th Int. Workshop, abstr., p. 27, 1998), hemolytic-uremic syndrome (7), and hypogammaglobulinemia (8) and opportunistic infections in immunocompromised hosts (25, 44). However, in contrast to *C. jejuni* (46), knowledge about the genomic characteristics of *C. upsaliensis* is limited, and its possible pathogenic capacity for humans and small animals is far from being well defined (4).

Genotypic methods have successfully been applied to accomplish phenotypic approaches to the subtyping of *Campylobacter* species (32, 45, 49, 54, 63). Previous reports of studies that used pulsed-field gel electrophoresis (PFGE) to characterize *C. jejuni* (21, 42, 65), *C. upsaliensis* (3, 34, 44), and *C. hyointestinalis* (52) at the genomic level demonstrated the use-

fulness of this method for epidemiological studies. Flagellin gene polymorphism (20, 37, 38) or the combination of macrorestriction analysis and serotyping based on heat-stable or heat-labile antigens have also been performed to distinguish *C. jejuni* strains from *C. coli* strains (43, 47, 54). Investigations of the genotypic and serological diversity of canine and feline *C. upsaliensis* strains originating from two different areas in Germany were previously performed in our laboratories. Human isolates were not available, since *C. upsaliensis* does not seem to be of major relevance as a human pathogen in Germany. Macrorestriction analysis as well as enterobacterial repetitive intergenic consensus sequence (ERIC) PCR (ERIC-PCR) profiling was used, and a serotyping scheme for heat-stable antigens was developed (34). In general, the study revealed a high degree of genomic diversity but a low degree of serological diversity. The genotypic clusters of the strains did not reflect their geographic origins in Germany or the health status of the carriers. As the vast majority of strains were isolated from dogs and only very few strains were isolated from cats and none was isolated from humans, host-specific differences could not be addressed in this investigation. In the present study *C. upsaliensis* strains isolated from humans, dogs, and cats in Canada and Australia were genotyped by the same methods described before (34) and compared with the German strains.

The aim of this study was to extend the knowledge of genomic characteristics of *C. upsaliensis* strains originating from different hosts and different areas of the world.

MATERIALS AND METHODS

Collection and cultivation of bacteria. Seventy *C. upsaliensis* strains were recovered from dogs ($n = 63$) and cats ($n = 7$) of different ages and enteric health conditions in two regions of Germany (Berlin and Northrhine-Westphalia) approximately 400 km apart. The isolates were collected from rectal swab spec-

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TABLE 1. Bacterial strains used for antiserum preparation

Bacterial strain	Species	Serotype	Origin and strain type
Ulr.3	<i>C. upsaliensis</i>	OI	Berlin, dog, wild-type strain
H 16	<i>C. upsaliensis</i>	OIII	Berlin, dog, wild-type strain
H 29	<i>C. upsaliensis</i>	OIV	Berlin, dog, wild-type strain
H 5	<i>C. upsaliensis</i>	OVI	Berlin, dog, wild-type strain
K1E	<i>C. helveticus</i>	OV	NRW, cat, wild-type strain
10935	<i>C. jejuni</i>	O:1	CCUG, reference strain
10936	<i>C. jejuni</i>	O:2	CCUG, reference strain
10938	<i>C. jejuni</i>	O:4	CCUG, reference strain
10945	<i>C. jejuni</i>	O:13	CCUG, reference strain
10947	<i>C. jejuni</i>	O:16	CCUG, reference strain
12783	<i>C. jejuni</i>	O:43	CCUG, reference strain
L2H	<i>C. jejuni</i>	O:37	Germany, wild-type strain, origin unknown
1834	<i>C. jejuni</i>	O:40	Germany, wild-type strain, human origin
Tu 429	<i>C. coli</i>	O:20	Germany, wild-type strain, porcine origin
Tu 440	<i>C. coli</i>	O:nt ^a	Germany, wild-type strain, porcine origin
68	<i>C. lari</i>	O:nt	Germany, wild-type strain, chicken origin

^a nt, nontypeable.

imens obtained by using Culturettes (Becton Dickinson). The isolates were transferred onto selective growth media within 24 h after collection. Three selective media, CAT agar (1), mCCD agar (23), and CSM agar (28), were used in order to obtain as many isolates as possible from the swabs. Further cultivation was performed on Mueller-Hinton agar containing 5% defibrinated sheep blood. The plates were incubated for 48 to 72 h under microaerophilic conditions at 39°C. The bacteria were stored as stock cultures in thioglycolate broth containing 15% glycerol at -70°C. Twenty Canadian *C. upsaliensis* strains were kindly supplied by F. Rodgers and L. Price, Winnipeg, Manitoba, Canada. Eighteen were of human origin, one was of canine origin, and one was of feline origin. Six Australian *C. upsaliensis* strains isolated from humans were kindly supplied by G. Hogg, Melbourne, Australia. German *C. upsaliensis* isolates of human origin were not available.

Species determination. *C. upsaliensis* isolates were identified by biochemical tests described in the literature (22, 24, 40, 41) by use of the following criteria: gram negative, spiral-shaped rod morphology, motility, requirement of microaerophilic growth conditions, cytochrome oxidase activity, weak or no catalase activity, lack of hippuricase activity, capacity to reduce nitrate, lack of H₂S production on triple sugar iron agar, selenite reduction, and sensitivity to nalidixic acid and cephalothin. Indoxyl acetate activity was tested in some but not all isolates. The results were confirmed by using the species-specific PCR of Eyers et al. (9). Only isolates that reacted by conventional and molecular tests as described in the literature were included in the study.

Indirect hemagglutination. Indirect hemagglutination was performed as described previously (34, 36, 50). Five heat-stable antigens specific for *C. upsaliensis* were determined by the serotyping scheme developed in our laboratories. In addition, the strains were tested with eight *C. jejuni*-specific antisera, two *C. coli*-specific antisera, and one *C. lari*-specific antiserum available in the laboratory. Serum hemagglutination titers of 1:80 and less were ignored. *C. jejuni* reference strains were purchased from the Culture Collection University of Göteborg (CCUG). The *C. jejuni* and *C. coli* wild-type strains used as reference strains were serotyped at CCUG. The bacterial strains used for antiserum production are listed in Table 1.

DNA preparation, primers, and PCR amplification. The DNA preparation and PCR amplification procedures were performed as described previously (9, 34, 58). Briefly, for PCR amplification DNA was extracted by heat treatment. The amplification reaction was performed with Ready-To-Go PCR Beads (Amersham Pharmacia Biotech, Freiburg, Germany). Primer sequences were deduced from the sequences of the 23S rRNA and 16S rRNA genes of thermophilic *Campylobacter* species (9, 31) and ERIC sequences (58), as described before (34). Each PCR included positive and negative controls. Amplified samples were analyzed by electrophoresis on 1.2% agarose gels and visualized by ethidium bromide staining under UV light. The gels were photographed with a digital camera system (Herolab, Wiesloch, Germany).

Macrorestriction analysis by PFGE. PFGE was performed with the endonuclease XhoI, as described before (34), by using a CHEF DR III apparatus (Bio-Rad,

Munich, Germany). The pulse interval was ramped from 0.3 to 12 s linearly for 24 h. Reference DNA of *C. upsaliensis* strain DSM 5365 digested with XhoI was run on each gel. Molecular size standard bacteriophage λ concatamers (molecular size, 48.5 kb; New England Biolabs, Frankfurt, Germany) were run on three lanes (both edges and the middle) of each gel. Finally, the gels were stained with ethidium bromide, viewed under UV light and photographed with Polaroid film.

Computational analysis. The electrophoretic patterns of the ERIC-PCR experiments were analyzed by using GelCompar II, version 2.50, software (Applied Maths BVBA, Kortrijk, Belgium; Herolab). Genetic similarities between isolates based on their positions and relative band areas were calculated by use of the algorithm of Ward (60) and the Pearson correlation coefficient (optimization, 0.0; tolerance, 0.0; for details, see the GelCompar II, version 2.50, manual). The following variable areas of the gel tracks were included in the analysis of genetic similarity: 20.5 to 56.6%, 62.6 to 65.7%, and 70.8 to 100%. Photographs of the gels from the PFGE experiments were scanned; and similarities were calculated by using the same software, algorithm, and coefficient, with a maximum tolerance of 1.0% and optimization of 0.5%. The reproducibility of the profiles was $\geq 94\%$. The jackknife statistical method (GelCompar II, version 2.50, software, for comparative analysis of electrophoresis patterns; Applied Maths) was applied to determine the significance of the defined similarity groups.

16S rDNA sequence analysis. A 1,275-bp DNA stretch of the 16S rRNA-encoding gene (16S rDNA) was amplified by PCR with primers Cups-1 (5'-CCC ATA CTC CTA TTT AGC AT-3') and Cups-2 (5'-GAT TCC ACT GTG GGG GA-3'), as described by Linton et al. (31). The DNA sequences of the PCR products were analyzed by AGOWA Gesellschaft für molekularbiologische Technologie (Berlin, Germany) for the German strains and by the Federal Research Centre for Virus Diseases of Animals (Bundesforschungsanstalt für Viruskrankheiten der Tiere, Jena, Germany) for the Canadian and Australian strains. Briefly, the 16S rDNA fragments were amplified with primers Cups-1 and Cups-2. Bands were excised from 1% agarose gels, and DNA was extracted by using the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany). Sequencing was performed as cycle sequencing with a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Darmstadt, Germany), according to the instructions of the manufacturer. Primers Cups-1, Cups-2, Cups-4 (5'-GCA GTA GGG AAT ATT GCG-3'), Cups-5 (5'-CGC TAA GGC GCG AAA GCG-3'), and Cups-6 (5'-CAA ATC AGC CAT GTT GCG G-3') were used for sequencing. Nucleotide sequences were determined with an ABI Prism 310 genetic analyzer (Applied Biosystems). The processing of the sequence data was conducted with the Vector NTI Suite, version 8.0, program (Informax Inc., Oxford, United Kingdom).

RESULTS

Distribution of heat-stable antigens. Ninety-four of 96 *C. upsaliensis* strains isolated in Germany, Canada, and Australia were typed on the basis of their heat-stable antigens by use of the serotyping scheme described previously (Table 2; 34). Two serotypes, serotypes OIII and OIV, were present in all three geographic groups at rates of 37.2 and 36.2%, respectively, and were

TABLE 2. Heat-stable antigen specificities of *C. upsaliensis* strains originating from Germany, Canada, and Australia

Serotype	Total	No. (%) of <i>C. upsaliensis</i> strains of the following geographic origin:		
		Australia ^a	Canada	Germany
OI	6 (6.4)	0	3 (15.0)	3 (4.4)
OII	1 (1.0)	0	0	1 (1.5)
OIII	35 (37.2)	4	7 (35.0)	24 (35.3)
OIV	34 (36.2)	1	8 (40.0)	25 (36.8)
OVI	3 (3.2)	0	0	3 (4.4)
Negative ^b	15 (16.0)	1	2 (10.0)	12 (17.6)
Total	94	6	20	68

^a As only six isolates from Australia were available, the percentage of serotypes among this group of strains was not calculated.

^b Negative with the available antisera.

FIG. 1. Genetic similarity based on ERIC-PCR profiles of *C. upsaliensis* strains from humans, dogs, and cats in Germany, Canada, and Australia.

present at the same orders of magnitude in the Canadian and the German strain collections (35.0 and 40.0%, respectively, versus 35.3 and 36.8%, respectively). The number of Australian strains tested ($n = 6$) was too small to calculate the proportion. Among the other serotypes, 6.4, 1.0, and 3.2% of the strains were serotypes OI, OII, and OVI, respectively. Of 94 *C. upsaliensis* isolates, 16.0% did not react with any *C. upsaliensis* antiserum. Beyond

this, heat-stable *C. upsaliensis* antigens did not react with antisera recognizing *C. helveticus* OV; *C. jejuni* O:1, O:2, O:4, O:13, O:16, O:37, O:40, or O:43 (Penner); *C. coli* O:20 or a non-cross-reacting *C. coli* strain; or one untyped *C. lari* strain available in the laboratory (34, 35, 36).

ERIC fingerprint analysis. All *C. upsaliensis* strains serotyped ($n = 94$), with six of the German strains tested in dupli-

TABLE 3. Internal stability (significance) of the defined strain clusters by geographic and host origin

Method and origin	% Correct identification					
	Germany	Canada	Australia	Human	Dog	Cat
ERIC PCR						
Germany	98.7	0	16.7			
Canada	0	94.7	0			
Australia	1.3	5.3	83.3			
XhoI macrorestriction						
Germany	96.5	10.0	50.0			
Canada	3.5	85.0	33.3			
Australia	0	5.0	16.7			
XhoI macrorestriction						
Human				75.0	5.9	12.5
Dog				25.0	88.2	62.5
Cat				0	5.9	25.0

cate, were characterized by ERIC-PCR (Fig. 1). Two main clusters that differed considerably with respect to band pattern similarity were generated (-80% on the Ward scale of -100% through $+100\%$). One cluster contained the German strains, and the other one contained the non-European strains. With an internal similarity of -50% , the German strains, the most numerous group in this study, exhibited remarkable intragroup heterogeneity. The two geographic regions of Germany, approximately 400 km apart, from which the strains were isolated were not reflected by the dendrogram. The non-European cluster consisted of the Canadian strains, which exhibited an internal similarity of -20% , and the Australian strains were integrated into this group at a $+40\%$ similarity. Only one German canine strain was included in the non-European cluster. With respect to host association, the most numerous German cluster contained canine and feline strains, whereas the non-European cluster consisted of all human strains, two canine strains, and one feline strains.

The results of statistical calculations assessing the stabilities (significance) of the defined groups are shown in Table 3, which presents the percentages of correct identifications for members of a group. German strains were grouped in the German cluster at a rate of 98.7%. Canadian strains were assigned to the Canadian cluster at a rate of 94.7%, while 5.3% of Canadian strains exhibited a higher degree of similarity to Australian strains. Of the Australian strains, 83.3% were classified in the Australian group.

Macrorestriction analysis. For DNA macrorestriction analysis, 50 of 63 canine strains of German origin were selected to ensure that the most prevalent serotypes and the untypeable strains were represented in comparable numbers. Isolates from all the other sources were included in the total. Therefore, 83 *C. upsaliensis* strains were subjected to macrorestriction analysis with the XhoI endonuclease.

In general, most of the isolates possessed a number of recognition sites for XhoI, yielding between 10 and 20 DNA bands (molecular sizes, up to approximately 250 kb; Fig. 2). Every isolate exhibited a unique restriction pattern, as shown in the dendrogram in Fig. 2. Two major PFGE groups that reflected each of the geographic and the host origins of the strains were differentiated. With regard to geographic origin, one group contained

German strains at a rate of 73% and non-European strains at a rate of 11.5%. The second group consisted of non-European strains at a rate of 89.5% and German strains at a rate of 27%. With regard to host origin, one group contained strains of animal origin at a rate of 78.0% and strains of human origin at a rate of 37.5%. The other group consisted of strains of animal origin at a rate of 22.0% and strains of human origin at a rate of 62.5%. There was no association of PFGE groups with serotypes. The stability of the defined groups (significance) is shown in Table 3. On the basis of geographic origin, 96.5% of the German strains were grouped in the German cluster and 85.0% of the Canadian strains were grouped in the Canadian cluster, but only 16.7% of the six Australian strains were classified in the Australian cluster. On the basis of host association, 75.0% of the human strains were grouped in the human cluster, 88.2% of the canine strains were assigned to the canine cluster, and 25.0% of the feline strains were classified in the feline cluster.

Five strains (Fig. 2, diamonds) were isolated from the blood and stool of a newborn girl in Canada and two pet animals living in the same household. The results for these five strains are presented separately in Fig. 3. Two strains, one isolated from the baby's stool and a canine strain, exhibited identical band patterns and serotypes (serotype OI). A second strain from the child's stool and a strain from the child's blood were characterized by identical serotypes (serotype OIV) that differed from those of the first two strains and similar but not identical band patterns. The differences were perhaps due to methodological problems during strain preparation. The feline strain was unique. Two other German feline strains (Fig. 2, dots) were isolated from littermates, and two canine strains (Fig. 2, left-pointing triangles) were isolated from animals living in one household.

16S rDNA sequence analysis. Among the 83 *C. upsaliensis* strains investigated by macrorestriction analysis, seven German, three Australian, and four Canadian strains (Fig. 2, right-pointing triangles) were selected for 16S rDNA sequence analysis. According to Linton et al. (31), the major part of the 16S rRNA-encoding DNA sequence was amplified. A DNA stretch of 1,169 nucleotides of each of the 14 strains beginning at nucleotide position 225 (64) (GenBank accession number L14628) was analyzed. This corresponded to 80.1% of the entire 16S rRNA gene of *C. upsaliensis* CCUG 14913, which comprises 1,460 nucleotides. The strains possessed identical nucleotide sequences compared to the sequence of strain CCUG 14913, with two minor variations. At position 377, the C in one Canadian strain, two Australian strains, and one German strain was replaced by G; and the G at position 382 was replaced by C in the same strains.

DISCUSSION

C. upsaliensis is a microorganism that is widespread on all continents and that is primarily isolated from the intestinal environment of dogs. Its relevance as a pathogen that causes enteric diseases in animals is not clear. However, on the basis of a number of reports from different countries, it is recognized as a human pathogen. The different frequencies of isolation from humans may be due to the methodological approaches, the need for supplements or intolerance to the antibiotics present in growth media, or different socioeconomic conditions in different countries. It may also be related to the develop-

FIG. 2. Genetic similarity based on the macrorestriction profiles of *C. upsaliensis* strains from humans, dogs, and cats in Germany, Canada, and Australia generated with the restriction endonuclease *Xho*I.

FIG. 3. PFGE band patterns (XhoI) of *C. upsaliensis* isolates from a newborn child, a dog, and a cat living in the same household in Canada.

ment of genomic characteristics of the microbe which cause differences in virulence in different areas and continents. The analysis of the *C. upsaliensis* strains from Germany, Canada, and Australia revealed a considerable degree of genomic heterogeneity. These data are in accordance with those from previous reports and may be due to the properties of *Campylobacter* strains which enable them to take up DNA from the environment and integrate it into the genome or undergo changes within the genome by rearrangement of DNA (4, 16, 20, 59, 61, 62). Heat-stable antigen typing revealed that, in contrast to *C. jejuni* and *C. coli*, which are divided into more than 60 heat-stable serotypes, as described by Penner and colleagues (50, 51), *C. upsaliensis* seems to possess only a small number of different heat-stable antigens. This is true not only for strains isolated in Germany but also for strains isolated in Canada and Australia. The difference in heat-stable (Penner-type) antigen diversity between *C. jejuni*-*C. coli* and *C. upsaliensis* may perhaps be due to different biochemical structures of the respective antigens. Karlyshev et al. (27) reported evidence that the antigens of *C. jejuni* accounting for the Penner serotype specificity are capsular structures. It must be determined whether the heat-stable antigens of *C. upsaliensis* are capsular or lipooligosaccharide-lipopolysaccharide in nature. Eighty-four percent of the strains belonged to five different serotypes, and the ratio of serotypes was comparable for all three continents. Two of the serotypes, serotypes OIII and OIV, were prevalent at rates between 35 and 40% in Germany and Canada. The number of Australian strains available for this study was too small to calculate percentages. However, the tendency for a serotypic prevalence was also seen among the Australian strains, with four of the six strains belonging to serotype OIII. Among the German and Canadian strains, serotypes OI, OII, and OVI were detected at rates of 1.5 to 15%. These serotypes were not identified among the few Australian strains. These data point toward the clonal expansion of *C. upsaliensis* and indicate a high degree of conservation of heat-stable antigens of the species during evolution. Between 10 and 17.7% of all strains analyzed were untypeable with the available *C. upsaliensis*-specific antisera. Reactivity with antisera recognizing a number of heat-stable antigens of other *Campylobacter* species was not detected for any of the *C. upsaliensis* strains isolated in Germany, Canada, or Australia. Antisera against *C. helveticus* OV (34), nine heat-stable *C. jejuni* antigens mainly representing serotypes prevalent worldwide, two *C. coli* antigens, and one *C. lari* antigen were included. As reported previously (29), South African *C. upsaliensis* strains reacted with antiserum against *C. coli* O:28. It would have been interesting to determine whether the strains investigated in this study also shared antigenic determinants with *C. coli* O:28.

Unfortunately, the strain or the respective antiserum was not available. Therefore, this question could not be answered. Geographic restrictions perhaps contribute to the different results.

Nevertheless, despite the negative results obtained in the present study, it is not surprising that some cross-reactivity exists between *C. upsaliensis* and other closely related bacterial species.

In contrast to serotyping, genotypic methods (ERIC-PCR and PFGE) revealed a high degree of genomic heterogeneity within the species *C. upsaliensis*. ERIC-PCR profile analysis demonstrated the discriminatory potential of this method, as described before (34, 57, 58). Beyond this, it divided the strains into two clearly distinct genotypic clusters, a Canadian-Australian (non-European) cluster and a German (European) cluster, whereas the local origins of German strains were not reflected by the dendrogram. The jackknife calculations confirmed the validity of the defined groups, with the limitation that the assignment of Australian strains to the respective group might not have been sufficiently valid, since only six strains were available for the study. With respect to host origin, it could be noticed that the non-European strains, regardless of animal or human origin, were assembled in one group and that the German strains, both canine and feline, with one exception (a canine strain), were assembled in the other. The serotypic groups did not correlate with the genotypic clusters. The results give rise to the suspicion that diversification of the species into serotypes developed before dispersal of the species to different continents. In comparison to a collection of 88 German *C. jejuni* strains characterized previously (35), the *C. upsaliensis* strains formed a separate phylogenetic cluster, despite their intraspecies heterogeneity.

In the tree determined by XhoI macrorestriction analysis, intraspecies clustering was also detected. Two clusters were generated: one of them contained 73% of the German strains and 11.5% of the non-European strains, and the second cluster consisted of 88.5% of the non-European strains and 27% of the German strains. With regard to host origin, one cluster contained 78.0% of strains of animal origin and 37.5% of strains of human origin. The second cluster consisted of 22.0% of animal origin and 62.5% of strains of human origin. Subclusters reflecting the local origins of the German strains were, again, not detected, and an association of genotypic clusters with serotypes was also not found. The significance of the defined groups was again confirmed by jackknife calculations, with the limitation that the assignment of Australian and feline strains to the respective groups might not have been sufficiently strong, since only six and seven strains, respectively, were available for the study. The findings of Stanley et al. (55), who identified genotypic differences between human and canine

strains by 16S rRNA ribotyping, could not be confirmed by the methods used in the present study.

The high degree of 16S rDNA sequence similarity among the 14 *C. upsaliensis* isolates sequenced was in marked contrast to the high degree of heterogeneity of the whole genome and confirmed the idea that all the strains belonged to one species. Among the whole 16S rDNA length of 1,169 nucleotides, only one nucleotide was changed at each of two positions, and these changes were identical in the four strains from three continents. With these exceptions, all 14 strains exhibited 16S rDNA sequences identical to that of *C. upsaliensis* strain CCUG 14913 supplied in the database (64).

Investigating *C. upsaliensis* isolates originating from different continents and hosts, we intended to analyze the differences in the distributions of genomic characteristics in bacterial populations. The methods used in this study enabled us to detect and describe differences in structural but not functional aspects. The phenomenon that bacterial populations from different continents possess similar antigenic characteristics points toward a common source early in evolution. The Canadian and Australian strains assembled in one genotypic cluster more or less strictly separated from the German strains and may have been distributed to their continents from a common source at a later time.

The possible significance of *C. upsaliensis* in human disease is increasingly realized. The findings that strains from different continents exhibit significant differences in their genomic characteristics may point toward the possibility of differences concerning pathogenicity or host adaptation. Further studies are necessary to confirm the significance of these results.

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REFERENCES

- Aspinall, S. T., D. R. A. Wareing, P. G. Hayward, and D. N. Hutchinson. 1993. Selective medium for thermophilic campylobacters including *Campylobacter upsaliensis*. *J. Clin. Pathol.* **46**:829–831.
- Baker, J., M. D. Barton, and J. Lanser. 1999. *Campylobacter* species in cats and dogs in South Australia. *Aust. Vet. J.* **77**:662–666.
- Bourke, B., P. M. Sherman, D. Woodward, H. Lior, and V. L. Chan. 1996. Pulsed-field gel electrophoresis indicates genotypic heterogeneity among *Campylobacter upsaliensis* strains. *FEMS Microbiol. Lett.* **143**:57–61.
- Bourke, B., V. L. Chan, and P. Sherman. 1998. *Campylobacter upsaliensis*: waiting in the wings. *Clin. Microbiol. Rev.* **11**:440–449.
- Burnens, A., B. Angéloz-Wick, and J. Nicolet. 1992. Comparison of *Campylobacter* carriage rates in diarrheic and healthy pet animals. *J. Vet. Med. B* **39**:175–180.
- Burnens, A., and J. Nicolet. 1992. Detection of *Campylobacter upsaliensis* in diarrheic dogs and cats, using a selective medium with cefoperazone. *Am. J. Vet. Res.* **53**:48–51.
- Carter, J. E., and N. Cimolai. 1996. Hemolytic-uremic syndrome associated with acute *Campylobacter* gastroenteritis. *Nephron* **74**:489.
- Chusid, M. J., D. W. Wortman, and W. M. Dunne. 1990. "*Campylobacter upsaliensis*" sepsis in a boy with acquired hypogammaglobulinemia. *Diagn. Microbiol. Infect. Dis.* **13**:367–369.
- Eyers, M., S. Chapelle, G. Van Camp, H. Goossens, and R. De Wachter. 1993. Discrimination among thermophilic *Campylobacter* species by polymerase chain reaction amplification of 23S rRNA gene fragments. *J. Clin. Microbiol.* **31**:3340–3343. (Erratum, **32**:1623, 1994.)
- Fox, J. G., K. O. Maxwell, N. S. Taylor, C. D. Runswick, P. Edmonds, and D. J. Brenner. 1989. "*Campylobacter upsaliensis*" isolated from cats as identified by DNA relatedness and biochemical features. *J. Clin. Microbiol.* **27**:2376–2378.
- Gaudreau, C., and F. Lamothe. 1992. *Campylobacter upsaliensis* isolated from a breast abscess. *J. Clin. Microbiol.* **30**:1354–1356.
- Goossens, H., B. A. J. Giesendorf, P. Vandamme, L. Vlaes, C. Van den Borre, A. Koeken, W. G. V. Quint, W. Blomme, P. Hanicq, D. S. Koster, H. Hofstra, J.-P. Butzler, and J. Van der Plas. 1995. Investigation of an outbreak of *Campylobacter upsaliensis* in day care centers in Brussels: analysis of relationships among isolates by phenotypic and genotypic typing methods. *J. Infect. Dis.* **172**:1298–1305.
- Goossens, H., B. Pot, L. Vlaes, C. Van den Borre, R. Van den Abbeele, C. Van Naeltens, J. Levy, H. Cogniau, P. Marbehan, J. Verhoef, K. Kersters, J.-P. Butzler, and P. Vandamme. 1990. Characterization and description of "*Campylobacter upsaliensis*" isolated from human feces. *J. Clin. Microbiol.* **28**:1039–1046.
- Goossens, H., L. Vlaes, M. De Boeck, B. Pot, K. Kersters, J. Levy, P. De Mol, J.-P. Butzler, and P. Vandamme. 1990. Is "*Campylobacter upsaliensis*" an unrecognized cause of human diarrhoea? *Lancet* **335**:584–586.
- Goossens, H., L. Vlaes, J.-P. Butzler, A. Adnet, P. Hanicq, S. N'Jufom, D. Massart, G. De Shrijver, and W. Blomme. 1991. *Campylobacter upsaliensis* enteritis associated with canine infections. *Lancet* **337**:1486–1487.
- Guerry, P., S. M. Logan, and T. Trust. 1988. Genomic rearrangements associated with antigenic variation in *Campylobacter coli*. *J. Bacteriol.* **170**:316–319.
- Gurgan, T., and K. S. Diker. 1994. Abortion associated with *Campylobacter upsaliensis*. *J. Clin. Microbiol.* **32**:3093–3094.
- Hald, B., and M. Madsen. 1997. Healthy puppies and kittens as carriers of *Campylobacter* spp., with special reference to *Campylobacter upsaliensis*. *J. Clin. Microbiol.* **35**:3351–3352.
- Hanna, J. N., R. M. Enbom, and D. M. Murphy. 1994. *Campylobacter upsaliensis* bacteraemia in an aboriginal child. *Med. J. Aust.* **160**:655–656.
- Harrington, C. S., F. M. Thomson-Carter, and P. E. Carter. 1997. Evidence for recombination in the flagellin locus of *Campylobacter jejuni*: implications for the flagellin gene typing scheme. *J. Clin. Microbiol.* **35**:2386–2392.
- Harrington, C. S., F. M. Thomson-Carter, and P. E. Carter. 1999. Molecular epidemiological investigation of an outbreak of *Campylobacter jejuni* identifies a dominant clonal line within Scottish serotype HS 55 populations. *Epidemiol. Infect.* **122**:367–375.
- Holländer, R. 1984. Characterization of *Campylobacter jejuni/coli*-isolates from human faeces. *Zentbl. Bakteriell. Parasitenkd. Infektkrankh. Hyg. Abt. 1 Orig. Reihe A* **258**:128–134.
- Hutchinson, D. N., and F. J. Bolton. 1984. Improved blood free selective medium for the isolation of *Campylobacter jejuni* from fecal specimens. *J. Clin. Pathol.* **37**:956–957.
- Hwang, M. N., and G. M. Ederer. 1975. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. *J. Clin. Microbiol.* **1**:114–115.
- Jenkin, G. A., and W. Tee. 1998. *Campylobacter upsaliensis*-associated diarrhea in human immunodeficiency virus-infected patients. *J. Clin. Infect. Dis.* **27**:816–821.
- Jimenez, S. G., R. G. Heine, P. B. Ward, and R. M. Robins-Browne. 1999. *Campylobacter upsaliensis* gastroenteritis in childhood. *Pediatr. Infect. Dis.* **18**:988–992.
- Karlyshev, A. V., D. Linton, N. A. Gregson, A. J. Lastovica, and B. W. Wren. 2000. Genetic and biochemical evidence of a *Campylobacter jejuni* capsular polysaccharide that accounts for Penner serotype specificity. *Mol. Microbiol.* **35**:529–541.
- Karmali, M. A., A. E. Simor, M. Roscoe, P. C. Fleming, S. S. Smith, and J. Lane. 1986. Evaluation of a blood-free, charcoal-based, selective medium for the isolation of *Campylobacter* organisms from feces. *J. Clin. Microbiol.* **23**:456–459.
- Lastovica, A. J., E. Le Roux, and J. L. Penner. 1989. "*Campylobacter upsaliensis*" isolated from blood cultures of pediatric patients. *J. Clin. Microbiol.* **27**:657–659.
- Lindblom, G.-B., E. Sjögren, J. Hansson-Westerberg, and B. Kaijser. 1995. *Campylobacter upsaliensis*, *C. sputorum sputorum* and *C. concisus* as common causes of diarrhea in Swedish children. *Scand. J. Infect. Dis.* **27**:187–188.
- Linton, D., F. Dewhirst, J. P. Cleweley, R. J. Owen, A. Burnens, and J. Stanley. 1994. Two types of 16S rRNA gene found in *Campylobacter helveticus*: analysis, applications and characterization of the intervening sequence found in some strains. *Microbiology* **140**:847–855.
- Lior, H., and D. L. Woodward. 1991. A serotyping scheme for *Campylobacter upsaliensis*. *Microb. Ecol. Health Dis.* **4**(special issue):89.
- Moreno, G. S., P. L. Griffiths, I. F. Connerton, and R. W. A. Park. 1993. Occurrence of campylobacters in small domestic and laboratory animals. *J. Appl. Bacteriol.* **75**:49–54.
- Moser, I., B. Rieksneuwöhner, P. Lentzsch, P. Schwert, and L. H. Wieler. 2001. Genomic heterogeneity and O-antigenic diversity of *Campylobacter upsaliensis* and *C. helveticus* strains isolated from dogs and cats in Germany. *J. Clin. Microbiol.* **39**:2548–2557.
- Moser, I., P. Lentzsch, B. Rieksneuwöhner, and P. Schwert. 2002. High resolution genotyping of *Campylobacter jejuni* strains by macrorestriction analysis with XhoI and polymerase chain reaction targeting enterobacterial repetitive intergenic consensus sequences: can we predict the zoonotic potential of strains? *Epidemiol. Infect.* **129**:435–443.
- Moser, I., and E. Hellmann. 1989. *In vitro* binding of *Campylobacter jejuni*

- surface proteins to murine small intestinal cell membranes. *Med. Microbiol. Immunol.* **178**:217–228.
37. Nachamkin, I., K. Bohachick, and C. M. Patton. 1993. Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *J. Clin. Microbiol.* **31**:1531–1536.
 38. Nachamkin, I., H. Ung, and C. M. Patton. 1996. Analysis of HL and O serotypes of *Campylobacter* strains by the flagellin gene typing system. *J. Clin. Microbiol.* **34**:277–281.
 39. Olson, P., and K. Sandstedt. 1987. *Campylobacter* in the dog: a clinical and experimental study. *Vet. Rec.* **121**:99–101.
 40. On, S. L. W. 1996. Identification methods for campylobacters, helicobacters, and related organisms. *Clin. Microbiol. Rev.* **9**:405–422.
 41. On, S. L. W., and B. Holmes. 1991. Effect of inoculum size on the phenotypic characterization of *Campylobacter* species. *J. Clin. Microbiol.* **29**:923–926.
 42. On, S. L. W., E. M. Nielsen, J. Engbert, and M. Madsen. 2000. Validity of SmaI-defined genotypes of *Campylobacter jejuni* examined by SalI, KpnI, and BamHI polymorphisms: evidence of identical clones infecting humans, poultry, and cattle. *Epidemiol. Infect.* **120**:231–237.
 43. Owen, R. J., C. Fitzgerald, K. Sutherland, and P. Borman. 1994. Flagellin gene polymorphism analysis of *Campylobacter jejuni* infecting man and other hosts and comparison with biotyping and somatic antigen serotyping. *Epidemiol. Infect.* **113**:221–234.
 44. Owen, R. J., and J. Hernandez. 1990. Genotypic variation in '*Campylobacter upsaliensis*' from blood and faeces of patients in different countries. *FEMS Microbiol. Lett.* **72**:5–10.
 45. Owen, R. J., K. Sutherland, C. Fitzgerald, J. Gibson, P. Borman, and J. Stanley. 1995. Molecular subtyping scheme for serotypes HS1 and HS4 of *Campylobacter jejuni*. *J. Clin. Microbiol.* **33**:872–877.
 46. Parkhill, J., B. W. Wren, K. Mungall, J. M. Ketley, C. Churcher, D. Basham, T. Chillingworth, R. M. Davies, T. Feltwell, S. Holroyd, K. Jagels, A. V. Karlyshev, S. Moule, M. J. Pallen, C. W. Penn, M. A. Quail, M.-A. Rajandream, K. M. Rutherford, A. H. M. van Vliet, S. Whitehead, and B. G. Barrell. 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* **403**:665–668.
 47. Patton, C. M., M. A. Nicholson, S. M. Ostroff, A. A. Ries, I. K. Wachsmuth, and R. V. Tauxe. 1993. Common somatic O and heat-labile serotypes among *Campylobacter* strains from sporadic infections in the United States. *J. Clin. Microbiol.* **31**:1525–1530.
 48. Patton, C. M., N. Shaffer, P. Edmonds, T. J. Barrett, M. A. Lambert, C. Baker, D. M. Perlman, and D. J. Brenner. 1989. Human disease associated with '*Campylobacter upsaliensis*' (catalase-negative or weakly positive *Campylobacter* species) in the United States. *J. Clin. Microbiol.* **27**:66–73.
 49. Patton, C. M., I. K. Wachsmuth, G. M. Evins, J. A. Kiehlbauch, B. D. Plikaytis, N. Troup, L. Tompkins, and H. Lior. 1991. Evaluation of 10 methods to distinguish epidemic-associated *Campylobacter* strains. *J. Clin. Microbiol.* **29**:680–688.
 50. Penner, J. L., and J. N. Hennessy. 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. *J. Clin. Microbiol.* **12**:732–737.
 51. Penner, J. L., J. N. Hennessy, and R. V. Congi. 1983. Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of thermostable antigens. *Eur. J. Clin. Microbiol.* **2**:378–383.
 52. Salama, S. M., H. Tabor, M. Richter, and D. E. Taylor. 1992. Pulsed-field gel electrophoresis for epidemiologic studies of *Campylobacter hyointestinalis* isolates. *J. Clin. Microbiol.* **30**:1982–1984.
 53. Sandstedt, K., J. Ursing, and M. Walder. 1983. Thermotolerant *Campylobacter* with no or weak catalase activity isolated from dog. *Curr. Microbiol.* **8**:209–213.
 54. Stanley, J., D. Linton, K. Sutherland, C. Jones, and R. J. Owen. 1995. High-resolution genotyping of *Campylobacter coli* identifies clones of epidemiologic and evolutionary significance. *J. Infect. Dis.* **172**:1130–1134.
 55. Stanley, J., C. Jones, A. Burnens, and R. Owen. 1994. Distinct genotypes of human and canine isolates of *Campylobacter upsaliensis* determined by 16S rRNA gene typing and plasmid profiling. *J. Clin. Microbiol.* **32**:1788–1794.
 56. Taylor, D. E., K. Hiratsuka, and L. Mueller. 1989. Isolation and characterization of catalase-negative and catalase-weak strains of *Campylobacter* species, including '*Campylobacter upsaliensis*,' from humans with gastroenteritis. *J. Clin. Microbiol.* **27**:2042–2045.
 57. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**:2233–2239.
 58. Versalovic, J., T. Koeuth, and J. R. Lupinski. 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res.* **19**:6823–6831.
 59. Wang, Y., and D. E. Taylor. 1990. Natural transformation in *Campylobacter* species. *J. Bacteriol.* **172**:949–955.
 60. Ward, J. H. 1963. Hierarchical grouping to optimize an objective function. *J. Am. Statist. Assoc.* **58**:236–244.
 61. Wassenaar, T. M., B. N. Fry, and B. A. M. van der Zeijst. 1995. Variation of the flagellin gene locus of *Campylobacter jejuni* by recombination and horizontal gene transfer. *Microbiology* **141**:95–101.
 62. Wassenaar, T. M., B. Geilhausen, and D. G. Newell. 1998. Evidence of genomic instability in *Campylobacter jejuni* isolated from poultry. *Appl. Environ. Microbiol.* **64**:1816–1821.
 63. Weijtens, M. J. B. M., J. van der Plas, P. G. H. Bijker, H. A. P. Urlings, D. Koster, J. G. van Logtestijn, and J. H. J. Huis in't Veld. 1997. The transmission of *Campylobacter* in piggeries; an epidemiological study. *J. Appl. Microbiol.* **83**:693–698.
 64. Wesley, I. V., L. Schroeder-Tucker, A. L. Baetz, F. E. Dewhirst, and B. J. Paster. 1995. *Arcobacter*-specific and *Arcobacter butzleri*-specific 16S rRNA-based DNA probes. *J. Clin. Microbiol.* **33**:1691–1698.
 65. Yan, W., N. Chang, and D. E. Taylor. 1991. Pulsed-field electrophoresis of *Campylobacter jejuni* and *Campylobacter coli* genomic DNA and its epidemiological application. *J. Infect. Dis.* **163**:1068–1072.