**Helicobacter sp. flexispira 16S rDNA taxa 1, 4 and 5 and Finnish porcine Helicobacter isolates are members of the species Helicobacter trogontum (taxon 6)**

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The term ‘flexispira’ refers to micro-organisms with a particular morphology: fusiform-shaped with helical periplasmic fibrils and bipolar tufts of sheathed flagella. Two flexispira taxa have been formally named, Helicobacter bilis and Helicobacter trogontum, a third named species is Helicobacter aurati and eight additional 16S rRNA sequence-based flexispira taxa have been described by Dewhirst et al. (Int J Syst Evol Microbiol 50, 1781–1787, 2000) and given the provisional designation Helicobacter sp. flexispira taxa 1–5, 7, 8 and 10. In the present study, seven gastric or intestinal flexispira isolates from seven Finnish pigs originating from different farms were characterized. Morphologically, all these porcine isolates had typical flexispira morphology. Analysis of the 16S rDNA sequences of five isolates showed that they were most closely related to the sequences of flexispira taxa 4 and 5 and *H. trogontum* (taxon 6), but less closely related to taxa 1–3 and 8, *H. bilis* and *H. aurati*. Phenotypic characterization, analysis of RFLPs of 16S and 23S rDNAs and SDS-PAGE profiles revealed that all of the porcine isolates, reference strains of flexispira taxa 1, 4 and 5 and the type strain of *H. trogontum* (ATCC 700114T) had highly related characteristics that differed from those of the reference strains of taxa 2, 3 and 8 and *H. bilis*. Furthermore, a high DNA–DNA binding rate was found, in dot-blot hybridization studies, between the Finnish porcine strains, taxa 1, 4 and 5 reference strains and *H. trogontum* ATCC 700114T. In conclusion, polyphasic characterization of novel porcine flexispira isolates and previously described taxa 1, 4 and 5 reference strains showed that they all belong to a validly described species, *H. trogontum*, and that the taxonomy of known flexispiras is less complicated than proposed on the basis of 16S rDNA sequence analysis.

**INTRODUCTION**

The taxonomy of the genus *Helicobacter* has been extended considerably during the last 10 years. At the time of writing, 20 species have been validly described (Melito et al., 2001; Solnick & Schauer, 2001; Robertson et al., 2001). *Helicobacter pylori* (*Campylobacter pylori*) was first isolated in 1982, and a valid description of the genus *Helicobacter* and of *H. pylori* as the first member of this genus was published in 1989 (Goodwin et al., 1989). *Helicobacter* species infect and colonize the gastrointestinal tracts of a large variety of hosts. Morphologically, they are large or small spiral rods, with or without periplasmic fibrils (Solnick & Schauer, 2001). A distinct group of spindle-shaped, motile micro-organisms with periplasmic fibres around the cells and bipolar tufts of sheathed flagella were provisionally named as ‘flexispira’ micro-organisms (Bryner, 1988). A flexispira strain was first isolated by Kirkbride et al. (1985) from aborted lambs, and ‘Flexispira rappini’ is the provisional name proposed for these isolates (Bryner, 1988). These micro-organisms belong to one of the three morphotypes described by Lockard & Boler (1970), who used transmission electron microscopy of samples of canine gastric mucosa. Several studies have shown that these micro-organisms are members of the genus *Helicobacter* (Lee et al., 1992; Schauer et al., 1993; Fox et al., 1995; Mendes et al., 1996; Dewhirst et al., 2000a). This morphological group includes three named species, *Helicobacter bilis* (Fox et al., 1995), *Helicobacter trogontum* (Mendes et al., 1996) and *Helicobacter aurati* (Patterson et al., 2000), respectively isolated from mice, rats and hamsters. *Helicobacter*

The GenBank accession numbers for the *Helicobacter* sp. flexispira 16S rDNA sequences obtained in this study are AY034817–AY034821, as detailed in Fig. 1.
muridarum also has external fibrils, but its cell morphology is spiral (Lee et al., 1992). In addition, flexispira-like microorganisms have been isolated from sheep, pigs and porcine foetuses (Kirkbride et al., 1985; Dewhirst et al., 2000a), from faecal and gastric samples from dogs (Romero et al., 1988; Eaton et al., 1996; Utriainen et al., 1997; Jalava et al., 1998) and from faecal material from humans with diarrhoea (Archer et al., 1988; Romero et al., 1988). Single isolates from the blood cultures of patients with bacteraemia have also been described (Tee et al., 1998; Sorlin et al., 1999; Weir et al., 1999). Recently, flexispiras were isolated from cotton-top tamarins (Saunders et al., 1999). Dewhirst et al. (2000a) performed phenotypic and phylogenetic analyses of 36 strains by using 16S rRNA sequence analysis: they divided the strains into 10 distinct taxa and suggested the provisional names Helicobacter sp. flexispira taxa 1–10 for the groups. The flexispira taxa had very similar phenotypic characteristics, but 16S rRNA sequence analysis showed them to be widely interspersed among intestinal Helicobacter species. Formal description of flexispira taxa 1–5, 7 or 8 was not possible either because the groups had only one or a few isolates for comparison (taxa 1–5 and 7) or because the phylotype contained more than one distinct phenotype (taxon 8) (Dewhirst et al., 2000a). Flexispira taxa 6 and 9 respectively correspond to the named species H. trogontum and H. bilis. H. aurati could be considered as flexispira taxon 11. The G+C content is 34 mol% for strain ATCC 43966 (=CCUG 28992) but is not known for other strains within the flexispira group (Solnick & Schauer, 2001).

In the present study, we show that a number of novel porcine isolates and previously described Helicobacter sp. flexispira taxa 1, 4 and 5 reference strains and H. trogontum (taxon 6) constitute a single species, revealing that ‘F. rappini’, the original name suggested by Bryner (1988) for an isolate from an aborted sheep foetus (ATCC 43966), is H. trogontum.

### METHODS

**Bacterial strains.** Flexispira-like micro-organisms were isolated either from the gastric mucosa of swine, sampled at the slaughterhouse, or from faecal samples of healthy piglets from two Finnish farms, collected between 1995 and 2000 (Table 1). Isolation was performed either on brucella blood agar with vancomycin, polymyxin B, trimethoprim and amphotericin B, nitrate membrane (0.65 μm pore size) on fresh brucella blood agar plates as described by Steele & McDermott (1984). The isolation procedure for three Finnish canine flexispira isolates used for comparison was similar to that for the porcine isolates. All media were incubated microaerobically in an atmosphere containing approximately 5% O2, 10% CO2, 3% H2 and 82% N2.

**Phenotypic characterization of the organisms.** Most tests were performed as described earlier (Hanninen et al., 1996; Jalava et al., 1997). In addition, growth was tested on brucella blood agar with 20% ox bile or 1% glycin, on brucella blood agar with 150 IU polymyxin B, on mCDA (Oxoid), on mCDA agar plates with 5–7% bovine blood and on brucella blood agar containing cefoperazone, amphotericin B and teicoplanin (CAT; Aspinall et al., 1996). All incubations were performed at 37°C.

### Table 1. Helicobacter strains used in this study

<table>
<thead>
<tr>
<th>Strain or isolate</th>
<th>Original designation</th>
<th>Source</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>Helicobacter trogontum</em></td>
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<td></td>
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<tr>
<td>Isolate HU 1SU</td>
<td>Pig, faeces</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>Isolate HU 2SU</td>
<td>Pig, faeces</td>
<td>This study</td>
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<td>This study</td>
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<tr>
<td>Isolate HU H158S</td>
<td>Pig, stomach</td>
<td>This study</td>
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<tr>
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<td>Pig, stomach</td>
<td>This study</td>
<td></td>
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<tr>
<td>Isolate HU 3SU</td>
<td>Pig, faeces</td>
<td>This study</td>
<td></td>
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<tr>
<td>Isolate HU A4</td>
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<td>This study</td>
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<td>ATCC 43968</td>
<td>Pig, faeces</td>
<td>Dewhirst et al. (2000a)</td>
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<tr>
<td>ATCC 49310</td>
<td>Sheep</td>
<td>Mendes et al. (1986)</td>
<td></td>
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<tr>
<td>CCUG 28992 (=ATCC 43966)</td>
<td>Aborted sheep foetus</td>
<td>Kirkbride et al. (1985, 1986); Bryner et al. (1987)</td>
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<tr>
<td>ATCC 700114T</td>
<td>LRB 8581T</td>
<td></td>
<td></td>
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<td><em>Other Helicobacter strains</em></td>
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<tr>
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<td>Sheep</td>
<td>Dewhirst et al. (2000a)</td>
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</tr>
<tr>
<td>ATCC 49320</td>
<td>Sheep</td>
<td>Dewhirst et al. (2000a)</td>
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<td>Romero et al. (1988)</td>
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<tr>
<td>CCUG 223435 (=ATCC 43879)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>H. bilis</em> ATCC 51630T</td>
<td>Taxon 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. hepaticus</em> CCUG 33637T</td>
<td>ATCC 51448T</td>
<td></td>
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<td>This study</td>
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<tr>
<td>Isolate KO 214</td>
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<td>Isolate KO 114</td>
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Electron microscopy. Transmission electron microscopy was performed for all Finnish flexispira isolates as well as for reference strains of flexispira taxa 1–5 and 8 and *H. bilis* (Table 1) by the methods described previously (Hänninen et al., 1996).

**SDS-PAGE.** SDS-PAGE protein profiles of the isolates were analysed from growth on brucella blood agar medium incubated for 2 days as described previously (Laemmli, 1970; Hänninen et al., 1996).

**Purification of DNA.** Bacteria were cultured on brucella blood agar plates for 2 days. The growth was collected from two to four plates and DNA was then isolated by the method of Pitcher et al. (1989) as described previously (Hänninen et al., 1996).

Whole DNA–DNA hybridization using the dot-blot technique. Purified DNA (50, 5 and 0.5 ng) was diluted and applied by filtration onto nylon membranes. Probes were prepared from DNA of the following strains: ATCC 43968 (flexispira taxon 1), ATCC 49314 (taxon 2), ATCC 49320 (taxon 3), ATCC 49310 (taxon 4), CCUG 38992 (taxon 5), ATCC 700114T (*H. trogontum*, taxon 6), CCUG 38994 (taxon 8), ATCC 700114T (*H. trogontum*, taxon 6), ATCC 51630T (*H. bilis*) and isolates HU H95S and HU 15U. The probes were labelled with digoxigenin with a DNA labelling and detection kit (Roche). The hybridization and all washings were performed at 58˚C as described previously (Hänninen et al., 1996; Jalava et al., 1998).

Amplification of 16S rRNA cistrons by PCR and purification of PCR products. 16S rRNA cistrons were amplified with bacterial universal primers F2 and F25 (Dewhirst et al., 1999). The PCR was performed in thin-walled tubes with a Perkin-Elmer 9700 thermocycler. One microlitre DNA template was added to a reaction mixture (50 μl final volume) containing 20 pmol each primer, 40 nmol dNTPs and 1 U *Taq* 2000 polymerase (Stratagene) in buffer containing Taqstart antibody (Sigma). In a hot-start protocol, samples were preheated at 95˚C for 8 min before amplification using the following conditions: denaturation at 95˚C for 45 s, annealing at 60˚C for 45 s and elongation for 1.5 min with an additional 5 s for each cycle. A total of 30 cycles were performed and then followed by a final elongation step at 72˚C for 10 min. The results of PCR amplification were examined by electrophoresis in a 1 % agarose gel. DNA was stained with ethidium bromide and visualised under short-wavelength UV light.

**16S rRNA sequencing.** Purified DNA from the PCR product was sequenced using an ABI Prism cycle-sequencing kit (BigDye Terminator cycle sequencing kit with AmpliTaq DNA polymerase FS; Perkin-Elmer). The sequencing primers were as described by Dewhirst et al. (2000a). Quarter dye chemistry was used with 80 μM primers and 1-5 μl PCR product in a final volume of 20 μl. Cycle sequencing was performed using an ABI 9700 sequencer, with 25 cycles of denaturation 96˚C for 10 s and annealing and extension at 60˚C for 4 min. Sequencing reactions were run on an ABI 377 DNA sequencer.

**16S rRNA data analysis.** Sequence data were entered into RNA, a program set for data entry, editing, sequence alignment, secondary structure comparison, similarity matrix generation and dendrogram construction for 16S rRNA in Microsoft QuickBasic for use with a PC, and were aligned as described previously (Paster & Dewhirst, 1988). Our database contains over 1000 sequences obtained in our laboratory and over 500 obtained from GenBank. Similarity matrices were constructed from the aligned sequences by using only those sequence positions for which 90 % of the strains had data. The similarity matrices were corrected for multiple base changes at single positions by the method of Jukes & Cantor (1969). Dendrograms were constructed by the neighbour-joining method (Saitou & Nei, 1987).

**PCR-RFLP of 23S rDNA and 16S rDNA.** For PCR-RFLP of 23S rDNA, purified DNA samples were amplified with primers L1 and L2 as described by Hurtado et al. (1997) and Jalava et al. (1999). Briefly, the amplified fragment of 2.7 kb was digested with four restriction enzymes, *Hae* III, *Hpa* II and *Hin* fI, as described by Hurtado et al. (1997) and Jalava et al. (1999). Similarly, PCR-RFLP of the 16S rDNA was performed as described by Riley et al. (1996), using *Helicobacter* genus-specific primers. The amplification products were digested with the restriction enzymes *Alu* I and *Mbo* I. The fragments were separated on 3 % agarose gels.

**RESULTS AND DISCUSSION**

**Phenotypic characterization**

The phenotypic characteristics of all porcine isolates were similar to those of the strains of flexispira taxa 1–5, *H. trogontum* and taxon 8. All the isolates and the reference strains of taxa 1, 4 and 5 were catalase- and urease-positive, had γ-galactamase transpeptidase activity, did not reduce nitrate to nitrite, grew at 37 and 42˚C but not at 25˚C and were resistant to nalidixic acid and cephalothin. *H. trogontum* has been characterized as a nitrate-non-reducing organism (Mendes et al., 1996). In most later descriptions, it was mistakenly described as a nitrate-reducing organism (e.g. Dewhirst et al., 2000a; Patterson et al., 2000; Solnick & Schauer, 2001). There was no growth of the isolates or of the reference strains of taxa 1–5, taxon 8 or *H. trogontum* on 1 % glycine brucella blood agar plates after 2–3 days incubation. None of the strains grew on brucella blood agar with 20 % bile salts. The bacteria did not grow on mCCDA, but when blood was included, growth occurred. The bacteria were resistant to 150 IU polymyxin B.

**Ultrastructure**

The most significant characteristic, which distinguishes the flexispira group from other *Helicobacter* species, is the morphology. The typical ultrastructure of isolate HU 15U is shown in Fig. 1. The ultrastructure within all of taxa 1, 4 and
5 was similar. The sizes of the cells varied from 3 to 7 μm by 0.5 to 0.8 μm. The most common size group was 4–5 μm. Typical periplasmic fibrils were seen around the fusiform cell body, and all isolates had sheathed flagella at both ends of the cells. The discrepancy in the description of *H. trogontum* and taxa 1 and 5 strains in the literature is in the number of flagella. Mendes et al. (1996) described the number of flagella as varying from five to seven and Dewhirst et al. (2000a) gave the number for taxa 1 and 5 as varying from 10 to 20. In our electron microscopy studies, we found a variable number of flagella, from six to 14. The variable numbers presented may be explained by methodological differences in different studies. In aged cultures, coccoid forms with periplasmic fibrils and flagella around the cells were visible.

### Protein patterns

SDS-PAGE protein patterns of selected isolates are shown in Fig. 2. Fig. 2(a) shows the overall similarity of banding patterns of reference strains of *Helicobacter* sp. *flexispira* taxa 1–5 and 8, *H. bilis* ATCC 51630T and *H. trogontum* ATCC 700114T. The banding patterns are similar in the area of major bands. Dense protein bands are located in the area corresponding to a molecular mass of approximately 50 kDa. Fig. 2(b) includes the banding patterns of porcine isolates HU 1SU, HU 2SU, HU H158S, HU H95S, HU 3SU and HU A4. The banding patterns of the isolates show overall similarity to those of the reference strains of *flexispira* taxa 4 and 5 (lanes 6 and 7, respectively). However, differences in the banding patterns existed, especially in the presence or absence of dense protein bands in the area corresponding to a molecular mass of approximately 50 kDa.

**Dot-blot hybridization**

Whole-genomic DNA–DNA hybridization experiments are important tests in the delineation of bacterial species (Wayne et al., 1987). Our study is the first in which DNA–DNA hybridization studies have been widely applied to studies on taxonomic relatedness within the *flexispira* group. Strong hybridization was found between the type strain of *H. trogontum*, ATCC 700114T, and the reference strains of taxa 1, 4 and 5 as well as with the porcine isolates HU 1SU, HU H95S, HU H158S, HU 3SU and HU A4. Weak hybridization was found between *H. trogontum* and the reference strains of *flexispira* taxa 2, 3 and 8, with two Finnish canine isolates as well as with *H. bilis* ATCC 51630T. Similarly, when the strains of taxa 1, 4 or 5 were used as probes, they hybridized strongly with the DNAs of *H. trogontum* and Finnish porcine isolates. These studies were repeated at least three times. Isolates HU H95S and HU 2SU used as probes also hybridized strongly with the reference strains of taxa 1, 4, 5 and with other porcine isolates, and less intensively with the strains from taxa 2, 3 and 8. When the reference strains of taxa 2, 3 and 8 and *H. bilis* ATCC 51630T were used as probes, only weak hybridization was found with the DNA of taxa 1, 4 and 5, *H. trogontum* and the porcine *flexispira* isolates. Weir et al. (1999) hybridized DNA from their blood-culture isolate (*flexispira* taxon 8) with that of strain ATCC 43966 (taxon 5) and found only 24 % relatedness. This is in agreement with our hybridization results.

**16S rRNA sequence analysis**

16S rDNA sequencing has been shown to be a powerful tool in phylogenetic studies of *Campylobacter* and *Helicobacter* species (Paster et al., 1988; On, 2001; Vandamme et al., 2000; Dewhirst et al., 2000b). Almost-complete 16S rRNA sequences (approx. 1490 bases) were determined for isolates *HU* 1SU, *HU* 2SU, *HU* 3SU, *HU* H95S and *HU* H157S. A phylogenetic tree based on 16S rDNA sequence analysis is presented in Fig. 3. 16S rDNA sequences used for building the tree are from 40 strains, the GenBank accession numbers of which are indicated in the tree. Pig isolates *HU* 2SU and *HU* H157S and *flexispira* taxon 4 strain ATCC 49310 had identical 16S rDNA sequences. The porcine isolates *HU* 3SU, *HU* H95S and *HU* 1SU were in the same cluster as *H. trogontum* ATCC 700114T and reference strains of taxa 4 and
5, and differed from the flexispira taxon 4 reference strain by up to 47 base differences. *H. tropontum* ATCC 700114T differed by 3.6 and 2.6%, respectively, from the reference strains of taxa 4 and 5. When Mendes et al. (1996) described *H. tropontum* as a novel *Helicobacter* species, they found 4.3% difference between the sequences of *H. tropontum* and ‘*F. rappini*’ ATCC 43966 (= NACD 1893, taxon 5). Later, Dewhirst et al. (2000a) found a discrepancy between the sequences of isolate NACD 1893 [received from J. H. Bryner and sequenced as ATCC 43966] and the original Kirkbride strain 84-3345 (received from the American Type Culture Collection, Manassas, VA, USA). In the present phylogenetic analysis, the corrected sequence of ATCC 43966 is used.

The 16S rDNA sequence of the *Helicobacter* sp. flexispira taxon 1 reference strain differed by 4.5% from the sequence of *H. tropontum*. However, our DNA–DNA hybridization data revealed that our porcine isolates, *Helicobacter* sp. flexispira taxa 1, 4 and 5 and *H. tropontum* ATCC 700114T have a high level of relatedness. Thus, the porcine isolates and the reference strains of taxa 1, 4 and 5 are actually members of the species *H. tropontum*, even though their 16S rRNA sequences differed considerably (by up to 4.5%).

There are several examples that indicate that 16S rDNA sequence similarity comparisons do not always provide conclusive evidence for species delineation; this is because we do not know the within-species diversity. Vandamme et al. (2000) found that the 16S rDNA sequences of *Helicobacter cinaedi* strains varied considerably (4.3% divergence), indicating that 16S rDNA sequence data can be misleading and cannot be used as the ‘gold standard’ for species determination. Similarly, Harrington & On (1999) showed that 16S rDNA sequences of *Campylobacter*

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**Fig. 3.** Neighbour-joining phylogenetic tree for *Helicobacter* sp. flexispira taxa and representative *Helicobacter* and *Campylobacter* species, based on 16S rRNA sequence comparisons. GenBank accession numbers are given in brackets. Sources of strains are also given.
hyointestinalis strains differed by up to 4.3%. In conclusion, 16S rDNA sequence similarity analysis is not a suitable taxonomic test for species delineation in the genus Helicobacter. In addition to DNA–DNA hybridization, multi-locus phylogenetic analysis of several conserved genes could be considered as a test for confirmation of the taxonomic relationships of uncharacterized Helicobacter strains. When Suerbaum et al. (2002) applied this technique and sequenced seven housekeeping genes and two flagellin genes for Helicobacter nemestrinae, they showed that this species is actually a member of H. pylori.

**PCR-RFLP of 16S and 23S rDNA sequences**

Digestion of 16S rDNA amplification products with AluI produced two patterns. Strains of flexispira taxa 1, 4 and 5, H. trogontum and all the porcine isolates had identical patterns (pattern 1), while all other strains studied, from taxa 2, 3, 8, 9 (H. bilis) and Helicobacter hepaticus, had pattern 2. MboI patterns of all strains, including porcine isolates, were identical (results not shown). We also used the RFLP of 23S rDNA as a supplementary tool in the identification of Helicobacter species, as proposed by Hurtado & Owen (1997) and Jalava et al. (1999) (Fig. 4). Digestion of 23S rDNA with the four enzymes HaeIII, HpalI, Hhal and Hinfl subdivided the porcine isolates, the strains of flexispira taxa 1–5 and 8, H. trogontum, H. bilis and three canine flexispira isolates into six types (Table 2). The patterns of H. trogontum ATCC 700114T (Fig. 4c) were identical to the HaeIII, Hpal and Hinfl patterns of the reference strain of taxon 5 (Fig. 4a, lane 10) and to those of the porcine isolates HU H95S (Fig. 4a, line 9), HU 3SU and HU A4, indicating high sequence similarity. Its Hhal pattern was identical to that of the taxon 1 reference strain (Fig. 4b, lane 1). The taxon 4 reference strain and isolates HU 1SU, HU 2SU, HU H158S and HU H157S had identical, or almost identical, HaeIII, Hhal (Fig. 4a, b) and Hinfl patterns. Reference strains of flexispira taxa 2 and 3 and the three Finnish canine isolates had different RFLP patterns and were grouped together in type T 2. Flexispira taxon 8 strains had a type T 4 pattern, H. bilis ATCC 51630T had a type T 5 pattern and H. hepaticus CCUG 33637T had a type T 6 pattern. None of the digestion enzymes alone was able to distinguish H. trogontum from other flexispira taxa: a combination of at least two enzymes is needed.

Our studies showed that the host spectrum of H. trogontum must be expanded from rats to include sheep and pigs (Kirkbride et al., 1985; Bryner et al., 1987; Mendes et al., 1996). Kirkbride et al. (1985, 1986) cultured spirillum-like organisms from aborted lambs with focal, hepatic necrosis during lambing seasons in 1984, 1985 and 1986, using an oxygen-free atmosphere and a selective culture medium developed for the isolation of Treponema hyodysenteriae (later Serpulina hyodysenteriae). When Kirkbride et al. (1986) inoculated their spirillum-like organisms intravenously

![Fig. 4.](image-url)
into pregnant ewes, these micro-organisms caused infection and abortion in three of 28 pregnant ewes. Later, abortion was caused in pregnant guinea pigs after intraperitoneal inoculation of the micro-organisms (Bryner et al., 1987). The latter authors also found antibodies against the spirillum in 19 of 30 inoculated guinea pigs. Taxon 1 reference strain ATCC 43968 was originally isolated from a pig in 1986, and taxon 4 reference strain ATCC 49310 was isolated from the liver of an aborted sheep in 1986 (Kirbride et al., 1985; Dewhirst et al., 2000a). H. trogontum was isolated from the colonic mucosa of laboratory rats (Mendes et al., 1996). Our porcine isolates were all from healthy animals, either piglets aged approximately 2 months or fattening pigs sampled at the slaughterhouse. The pathogenicity of ‘F. rappini’ (= H. trogontum) for sheep has been confirmed (Kirkbride et al., 1986; Bryner et al., 1987), but the pathogenicity for other hosts remains unknown.

In conclusion, by means of a polyphasic approach (Vandamme et al., 1996) together with the recommended criteria for the description of novel Helicobacter species (Dewhirst et al., 2000b), we identified the provisional taxa 1, 4 and 5 reference strains and Finnish porcine flexispira isolates as members of H. trogontum. H. trogontum is an example of a bacterial taxon with highly divergent 16S rDNA sequences. Our results make the present taxonomy of Helicobacter species with flexispira morphology more consistent and emphasize the benefit of the use of polyphasic studies including DNA–DNA hybridization in the characterization and description of novel Helicobacter species. The taxonomy of Helicobacter sp. flexispira taxa 2, 3, 7 and 8 is unclear and requires further study.

### ACKNOWLEDGEMENTS

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