Helicobacter cynogastricus sp. nov., isolated from the canine gastric mucosa

K. Van den Bulck,^{1†} A. Decostere,¹ M. Baele,¹ P. Vandamme,² J. Mast,³ R. Ducatelle¹ and F. Haesebrouck¹

Correspondence

K. Van den Bulck
kvdbulck@prdbe.jnj.com

¹Department of Pathology, Bacteriology and Poultry Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

²Department of Biochemistry, Physiology and Microbiology, Faculty of Sciences, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium

³CODA–CERVA–VAR, Groeselenberg 99, B-1180 Brussels, Belgium

A Gram-negative, microaerophilic helical rod, isolated from the gastric mucosa of a dog and designated strain JKM4^T, was subjected to a polyphasic taxonomic study. The tightly coiled organism, measuring 10–18 µm long and up to 1 µm wide, was motile by means of multiple sheathed flagella located at both ends of the cell and by a periplasmic fibril running along the external side of the helix. Strain JKM4^T grew preferably on biphasic culture plates or on very moist agar. Coccoid forms predominated in cultures older than 4 days as well as in growth obtained on dry agar plates. The strain grew at 30 and 37 °C, but not at 25 or 42 °C and exhibited urease, oxidase and catalase activities. On the basis of 16S rRNA gene sequence analysis, the novel isolate was identified as a member of the genus *Helicobacter* and showed > 97 % similarity to *Helicobacter felis*, *Helicobacter bizzozeronii* and *Helicobacter salomonis*, three species previously isolated from the canine gastric mucosa. Protein profiling of strain JKM4^T using SDS-PAGE revealed a pattern different from those of other *Helicobacter* species of mammalian gastric origin and from *Helicobacter canis*. Additionally, the urease gene sequence of strain JKM4^T was different from those of urease genes of *H. felis*, *H. bizzozeronii*, *H. salomonis* and '*Candidatus Helicobacter heilmannii*'. It is thus proposed that strain JKM4^T (=LMG 23188^T) represents a novel species within this genus, *Helicobacter cynogastricus* sp. nov.

Helicobacter species are fastidious and slow-growing organisms with specific nutritional and environmental requirements. Three *Helicobacter* species have previously been cultured from the gastric mucosa of cats and dogs, namely *Helicobacter felis*, *Helicobacter bizzozeronii* and *Helicobacter salomonis* (Hänninen *et al.*, 1996; Jalava *et al.*, 1997; Lee *et al.*, 1988). These three organisms are highly related to each other, both genetically and morphologically, although small morphological differences are perceptible through electron microscopic examination. The thicker and medium-length organism *H. felis* possesses two or three periplasmic fibrils wrapped around the cell body, while both *H. bizzozeronii*,

thinner and longer, and *H. salomonis*, thick, short and less spiralled, lack such appendages (Utriainen *et al.*, 1997). Recently, an uncultured spiral organism, '*Candidatus Helicobacter heilmannii*', was described as occurring in the gastric mucosa of one human and several wild felines (O'Rourke *et al.*, 2004) and it is most probably also present in the gastric mucosa of pet dogs and cats (Van den Bulck *et al.*, 2005a). In addition, less spiralled bacteria with numerous periplasmic fibrils, tentatively named '*Flexispira rappini*', have been isolated from the stomachs of various dogs (Hänninen *et al.*, 2003).

A study set up to determine the prevalence of each individual *Helicobacter* species in the canine and feline stomach led to the isolation of a large spiral organism from the stomach of a dog. In this study, it is suggested that this isolate represents a novel species of the genus *Helicobacter*.

The strain, designated JKM4^T, was isolated from the antrum region of the stomach of a euthanized dog at the Faculty of Veterinary Medicine, Ghent University, Belgium. Tissue samples were handled as described by Gruntar *et al.* (2003). Bacteria were grown on brain heart infusion (BHI; Oxoid)

†Present address: Janssen Pharmaceutica N.V., Global Preclinical Development, Turnhoutseweg 30, B-2340 Beerse, Belgium.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JKM4^T is DQ004689.

A phylogenetic tree based on 16S rRNA gene sequences for 27 *Helicobacter* strains and TEM images of strain JKM4^T are available as supplementary figures in IJSEM Online. A supplementary table detailing the 16S rRNA gene sequence similarity between *Helicobacter* strains is also available.

agar containing 10 % (v/v) horse blood, 5 mg amphotericin B l^{-1} (Fungizone; Bristol-Myers Squibb), *Campylobacter*-selective supplement (Skirrow; Oxoid) (containing 10 mg vancomycin l^{-1} , 5 mg trimethoprim lactate l^{-1} and 2500 U polymyxin B l^{-1}), Vitox supplement (Oxoid) and *Campylobacter* growth supplement (MAST; Lucron Bioproducts). Plates were incubated with lids uppermost at 37 °C under humidified microaerobic conditions in a closed circuit that was created by evacuating 80 % of the normal atmosphere and introducing a gas mixture of 8 % CO₂, 8 % H₂ and 84 % N₂.

Plates were checked every 2 days and BHI broth was added to the agar surface to ensure that the plates did not dry out. Primary growth occurred after 10 days of incubation as an oily aspect on the broth covering the agar medium. Examination of the broth by light microscopy revealed the presence of spiral-shaped, motile cells. Gram-staining revealed that the isolate was Gram-negative and had a helical shape. Growth of subcultures occurred as a spreading layer on moist agar plates. Pinpoint colonies were observed when an abundant amount of bacteria was inoculated onto a dry agar surface; however, bacteria grown on such a medium mostly lost their spiral morphology and transformed to coccoid forms.

Bacteria with typical spiral morphology were harvested in BHI broth and stored at -70 °C in a medium consisting of 7.5 g glucose, 25 ml BHI and 75 ml sterile inactivated horse serum.

Genomic DNA of strain JKM4^T was extracted using the DNeasy tissue kit (Qiagen) according to the manufacturer's instructions. To determine whether the novel isolate was a member of the genus *Helicobacter*, a *Helicobacter* genus-specific PCR was applied, as described previously by Germani *et al.* (1997). The positive result in this PCR demonstrated that strain JKM4^T represents a *Helicobacter* species.

To identify strain JKM4^T, a multiplex PCR was performed that enables the identification of *Helicobacter* species previously detected in the gastric mucosa of cats and dogs, namely *H. felis*, *H. bizzozeronii* and *H. salomonis* (Jalava *et al.*, 1998; Van den Bulck *et al.*, 2005a). This PCR is based on a part of the tRNA intergenic spacer of *Helicobacter* species, amplified with TET-labelled primers, and on the urease gene of *H. felis* (NED-labelled primers) and *H. bizzozeronii* (HEX-labelled primers), as described earlier (Baele *et al.*, 2004). DNA extracted from pure cultures of *H. felis*, *H. salomonis* and *H. bizzozeronii* served as positive controls, while highly purified water was included as a negative control. Fluorescently labelled PCR products were separated by means of capillary electrophoresis using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Product lengths were determined by interpolation with an internal size standard mixture of GeneScan 500 ROX (Applied Biosystems) and GeneScan 400-HD ROX (Applied Biosystems) using GeneMapper (Applied Biosystems).

Analysis of the PCR products produced from the DNA of strain JKM4^T in the multiplex PCR consistently revealed a TET-labelled amplicon of 136.6 bp, which differed from the TET-labelled amplicons of *H. felis* (137 bp), *H. bizzozeronii* (136 bp) and *H. salomonis* (134 bp). Additionally, no amplicons were obtained with the NED- or HEX-labelled primers for the urease gene of *H. felis* or *H. bizzozeronii*, respectively. These results indicated that strain JKM4^T is different from *H. salomonis*, *H. felis* and *H. bizzozeronii*.

'*Candidatus H. heilmannii*' is a hitherto unculturable spiral organism that is highly related phylogenetically to *H. felis*, *H. salomonis* and *H. bizzozeronii* and may occur in the gastric mucosa of wild carnivores. To exclude the possibility that strain JKM4^T was identical to '*Candidatus H. heilmannii*', DNA of strain JKM4^T was subjected to a PCR which specifically amplifies a part of the urease gene of this *Candidatus* organism (O'Rourke *et al.*, 2004). DNA from '*Candidatus H. heilmannii*' served as positive control, while highly purified water was included as a negative control. PCR products were separated by gel electrophoresis as described by Baele *et al.* (2004). PCR products obtained for '*Candidatus H. heilmannii*' and strain JKM4^T were sequenced using the BigDye terminator cycle sequencing kit (Perkin Elmer, Applied Biosystems) on the Genetic Analyzer. The electropherograms were exported and converted to KODON (Applied Maths) and sequences were aligned with GenBank using BLAST.

The '*Candidatus H. heilmannii*'-specific PCR resulted in the production of a 320 bp fragment for strain JKM4^T which consistently differed from the 380 bp fragment produced from DNA of '*Candidatus H. heilmannii*'. Sequence analysis of the 320 bp fragment of strain JKM4^T revealed a unique sequence bordered by the primer pair and the sequence obtained was not related to any sequence in GenBank.

The 16S rRNA gene of strain JKM4^T was amplified using primers complementary to the conserved edges. Consensus primers $\alpha\beta$ -NOT (5'-TCAAACCTAGGACCGAGTC) and ω MB (5'-TACCTTGTTACTTCACCCCA) were used, as described by Baele *et al.* (2001). A 1500 bp amplicon amplified in this PCR was sequenced using primers pD, γ^* , 3 and O*, as described elsewhere (Coenye *et al.*, 1999). Sequence analysis was performed using the Genetic Analyzer and sequences were aligned with GenBank using BLAST. Phylogenetic analysis was performed using the KODON program. Pairwise alignment similarities were calculated and a dendrogram was constructed using the neighbour-joining method.

The sequence of the 16S rRNA gene of strain JKM4^T showed > 97 % similarity to those of *H. felis*, *H. bizzozeronii*, *H. salomonis* and '*Candidatus H. heilmannii*' and the sequence differed by more than 3 % from that of '*Candidatus Helicobacter suis*' (see Supplementary Table S1 in IJSEM Online). A phylogenetic tree based on the 16S rRNA gene sequences revealed clustering of the novel isolate among *Helicobacter* species of carnivore origin (see Supplementary Fig. S1 in IJSEM Online).

Analysis of the urease gene has recently been found to be more discriminatory for species differentiation within gastric *Helicobacter* species of carnivore origin than 16S rRNA gene sequence analysis (O'Rourke *et al.*, 2004). Therefore, a PCR with primers U430f and U1735r was performed to amplify a part of the urease gene, as described previously (O'Rourke *et al.*, 2004).

For *H. felis*, *H. bizzozeronii*, *H. salomonis* and 'Candidatus *H. heilmannii*', a PCR amplicon of 1224 bp was obtained, while genomic DNA of strain JKM4^T produced a series of aspecific fragments instead of the expected 1224 bp fragment. The result confirms that strain JKM4^T is different from *H. felis*, *H. salomonis*, *H. bizzozeronii* and 'Candidatus *H. heilmannii*'.

PAGE of whole-cell proteins of strain JKM4^T was performed in order to establish its relationship with other cultured *Helicobacter* species. For this purpose, strain JKM4^T was grown on Mueller–Hinton agar supplemented with 5% (v/v) horse blood and incubated at 37 °C in a microaerobic atmosphere as described above. A whole-cell protein extract was prepared and SDS-PAGE was performed as described previously (Pot *et al.*, 1994). Whole-cell protein profiles of reference strains of *H. bizzozeronii*, *H. salomonis* and *H. felis* and of type and reference strains of other *Helicobacter* species were available from previous studies (Jalava *et al.*, 1998, 2001). Densitometric analysis, normalization and interpolation of the protein profiles and numerical analysis were performed using the GelCompar software package version 4.2 (Applied Maths). The similarity between all pairs of traces was expressed by the Pearson product-moment correlation coefficient, presented as percentages of similarity.

The whole-cell protein profile of strain JKM4^T differed considerably from those of the reference strains of other

Helicobacter species. Correlation levels to the protein profiles of other *Helicobacter* reference strains were all below 80%. Therefore, we conclude that strain JKM4^T represents a novel *Helicobacter* species. Whole-cell protein electrophoresis is a taxonomic tool that generally allows closely related species to be distinguished (Vandamme *et al.*, 1996). For many *Campylobacter* and *Helicobacter* species, the correlation between whole-cell protein pattern similarity and the level of DNA–DNA hybridization has been documented (Costas *et al.*, 1990; Jalava *et al.*, 1998, 2001; On *et al.*, 1995; Vandamme *et al.*, 1989, 1990, 1991, 1992a, b, 1997, 2000), even for species such as *Helicobacter cinaedi* and *Campylobacter hyointestinalis*, where substantial 16S rRNA gene sequence diversity has been observed (Vandamme *et al.*, 2000). The correlation between protein pattern similarity and the level of DNA–DNA hybridization has also been demonstrated for the phylogenetic cluster comprising *H. bizzozeronii*, *H. felis* and *H. salomonis* (Jalava *et al.*, 1998, 2001). Whole-cell protein electrophoresis has therefore been recommended as an alternative method for taxonomic discrimination at the species level in the recommended minimal standards for describing novel *Helicobacter* species (Dewhirst *et al.*, 2000). The result of the numerical analysis of the protein profiles of strain JKM4^T and its nearest cultured phylogenetic neighbours *H. bizzozeronii*, *H. salomonis* and *H. felis* is shown in Fig. 1.

The morphology of strain JKM4^T was studied by means of scanning and transmission electron microscopy (SEM, TEM). For these examinations, bacterial cultures were negatively stained (Houf *et al.*, 2005) or they were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in cacodylate buffer (0.1 M, pH 7.3). They were post-fixed in 1% (w/v) osmium tetroxide in distilled water. Samples for SEM were dehydrated in alcohol and acetone for subsequent

Fig. 1. Dendrogram derived from the numerical analysis of the whole-cell protein profiles of strain JKM4^T, *H. pylori*, *H. acinonychis*, *Helicobacter mustelae*, *Helicobacter aurati*, *Helicobacter muridarum*, *H. canis*, *H. bizzozeronii*, *H. salomonis* and *H. felis* reference strains. The grey bar in the marker lane indicates the part of the protein profile that was used in the numerical analysis.

critical-point drying in liquid CO₂, glued with carbon cement on to aluminium stubs, sputtered with a gold layer and examined with a Philips 501 SEM. Samples for TEM were block-stained with 2 % (w/v) uranyl acetate in distilled water and dehydrated in ethanol. They were embedded in Epon–Spurr's (1:1) medium. Ultrathin sections were cut from samples in which bacteria were demonstrated, stained with lead citrate and examined with a Philips EM 208S.

Cells of strain JKM4^T were large spiral cells, 10–18 µm long and approximately 1 µm wide, with three to eight spirals per cell (see Supplementary Fig. S2 in IJSEM Online). The presence of sheathed flagella located slightly off-centre at both ends of the cells demonstrated the morphological resemblance of strain JKM4^T to *H. salomonis*, *H. bizzozeronii* and *H. felis* (Utriainen *et al.*, 1997). The flagella of strain JKM4^T were blunt-ended and the terminal diameter was wider than the mean diameter of the flagellar body. Whereas periplasmic fibrils are thought to be a characteristic feature of *H. felis* (Utriainen *et al.*, 1997), a periplasmic fibril running along the external side of the helix was detected on every cell of strain JKM4^T. Coccoid forms were observed in cultures older than 4 days, as are also found in older cultures of *H. salomonis*, *H. bizzozeronii* and *H. felis*. Ultrastructural characteristics were examined several times after several subcultures and were the same in all studies.

Biochemical and tolerance tests were carried out as recommended by Dewhirst *et al.* (2000). The novel isolate was tested for oxidase, catalase (with 3 % hydrogen peroxide) and rapid urease activities and for hydrolysis of indoxyl acetate. The strain was also subjected to API Campy test strips (bioMérieux), which include tests for urease activity, nitrate reductase activity, esterase activity, hippurate hydrolysis, γ-glutamyl transpeptidase activity, alkaline phosphatase activity, triphenyltetrazolium chloride (TTC) reduction, pyrrolidonyl arylamidase and L-arginine and L-aspartate

arylamidase activities. Tolerance of 1 % glycine (Merck) and 1.5 % NaCl (Merck) was tested on tryptic blood agar base (Oxoid) supplemented with 10 % horse blood, as recommended by the Cape Town protocol for *Campylobacteriaceae* and helicobacters. Tolerance of ox bile was tested by plating the bacteria on unsalted MacConkey agar (Oxoid). Growth was tested on BHI blood agar, *Brucella* blood agar (Oxoid) and Mueller–Hinton blood agar. Growth at 25, 30, 37 and 42 °C was determined on BHI blood agar. All media were incubated for 7 days in a microaerobic atmosphere at 37 °C. In addition, growth on blood-supplemented BHI agar was tested in aerobic, aerobic with 5 % CO₂, microaerobic and anaerobic atmospheres.

The salient tests that distinguish the novel isolate from other gastric helicobacters and from *H. canis* are listed in Table 1, while the other biochemical and growth characteristics are given in the description below.

Susceptibility to metronidazole, ampicillin, clarithromycin, tetracycline, enrofloxacin, lincomycin, tylosin, neomycin, spectinomycin and gentamicin was tested on Mueller–Hinton agar (Oxoid) supplemented with 10 % horse blood using the agar dilution method as previously described (Van den Bulck *et al.*, 2005b). All antimicrobials were supplied by Sigma as standard powders with known potencies, except for enrofloxacin which was purchased from Bayer. These tolerance test preparations were incubated for 7 days in a microaerobic atmosphere at 37 °C. The novel isolate was susceptible to all antimicrobials tested, as indicated by low MIC values, ranging from 0.03 to 0.25 µg ml⁻¹.

Although until now only *H. felis*, *H. bizzozeronii* and *H. salomonis* have been isolated from the gastric mucosa of dogs, evidence has been presented previously that indicates that a significant proportion of the spiral organisms in the canine and feline stomach is unculturable (Cattoli *et al.*,

Table 1. Differential characteristics of *H. cynogastricus* JKM4^T sp. nov. and other *Helicobacter* species

Species: 1, *H. cynogastricus* sp. nov. (data from this study); 2, *H. felis* (Jalava *et al.*, 1997); 3, *H. bizzozeronii* (Jalava *et al.*, 1997); 4, *H. salomonis* (Jalava *et al.*, 1997); 5, *H. pylori* (Jalava *et al.*, 1997); 6, *H. acinonychis* (Eaton *et al.*, 1993); 7, *H. aurati* (Patterson *et al.*, 2000); 8, *H. muridarum* (Lee *et al.*, 1992); 9, *H. mustelae* (Fox *et al.*, 1988); 10, *H. canis* (Stanley *et al.*, 1993). +, Positive; –, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10
Cell length (µm)	10–18	5–7.5	5–10	5–7	2.5–5	1.2–2	4–8	3.5–5	2	3–4
Cell width (µm)	0.8–1.0	0.4	0.3	0.8–1.2	0.5–1.0	0.3	0.6	0.5–0.6	0.5	0.25–0.5
Periplasmic fibrils	+	+	–	–	–	–	+	+	–	–
Location of flagella	Bipolar	Bipolar	Bipolar	Bipolar	Polar	Polar	Bipolar	Bipolar	Lateral polar	Bipolar
No. of flagella	6–12	14–20	10–20	10–23	4–8	2–5	7–10	10–14	10–15	2
Catalase	+	+	+	+	+	+	+	+	+	–
Urease	+	+	+	+	+	+	+	+	+	–
Nitrate reduction	+	+	+	+	–	–	–	–	+	–
Indoxyl acetate hydrolysis	–	–	+	+	–	+	+	+	+	+
Alkaline phosphatase	+	+	+	+	+	+	–	+	+	+
Growth at 42 °C	–	–	+	–	–	+	+	–	+	+

1999; Jalava *et al.*, 1998). The present study demonstrates the existence of a fourth cultured *Helicobacter* species able to colonize the canine stomach, for which we propose the name *Helicobacter cynogastricus* sp. nov.

Following discussions held at the meetings of the International Committee on Systematics of Prokaryotes (ICSP) and its Judicial Commission (JC) in San Francisco in 2005, and in anticipation of the published minutes of the JC and ICSP, a committee consisting of the Chairman of the ICSP, the Chairman of the JC of the ICSP and the Editor of the *International Journal of Systematic and Evolutionary Microbiology* has granted an exception in this instance to Rule 27(3) of the *Bacteriological Code* governing the deposit of type material in two different collections in two different countries. *Helicobacter cynogastricus* sp. nov. JKM4^T has been submitted to the CCUG and NCTC culture collections but, due to the extremely fastidious nature of *Helicobacter* species, the strain has not been able to be revived or stored after lyophilisation. Attempts are still underway to complete the deposition of the type strain in a second culture collection.

Description of *Helicobacter cynogastricus* sp. nov.

Helicobacter cynogastricus (cy.no.gas'tri.cus. N.L. gen. n. cyno dog, from Gr. n. *kyon* kyno dog; N.L. masc. adj. *gastricus* of the stomach, from Gr. n. *gaster* stomach; *cynogastricus* N.L. masc. adj. of a dog's stomach).

Cells are tightly coiled spirals that are up to 1 µm wide by 10–18 µm long. They possess one periplasmic fibril running along the external side of the helix. In older cultures, coccoid cells predominate. Cells are Gram-negative and non-sporulating. They are motile by means of tufts of 6–12 sheathed flagella at one or both ends of the cell with a movement similar to that of *H. felis* and *H. bizzozeronii*. Growth on moist agar plates occurs as a spreading film or as an oily layer on biphasic culture media in a microaerobic and anaerobic atmosphere. Pinpoint colonies may be formed on dry agar plates, although bacteria are transformed into coccoids. Grows at 30 and 37 °C, but not at 25 or 42 °C. Does not grow on media containing 1 % ox bile, 1 % glycine or 1.5 % NaCl. Oxidase-, catalase- and urease-positive. Reduces nitrate and TTC and tests positive for esterase, γ-glutamyl transpeptidase, L-arginine arylamidase and alkaline phosphatase. Negative results are obtained in tests for hippurate and indoxyl acetate hydrolysis and for pyrrolidonyl arylamidase and L-aspartate arylamidase activities. The clinical significance of *H. cynogastricus* is unknown.

The type strain, JKM4^T (= LMG 23188^T), was isolated from the gastric mucosa of a dog.

Acknowledgements

This work was supported by the Research Fund of Ghent University, Belgium, Code GOA12050602. The authors are very grateful to Jurgen De Craene for his excellent technical assistance and to Dr Jani

O'Rourke for providing DNA of '*Candidatus* *H. heilmannii*' as a positive control in PCR tests. We also thank Stefanie Van Trappen and Emly Samyn for preparing the protein extracts.

References

- Baele, M., Devriese, L. A. & Haesebrouck, F. (2001). *Lactobacillus agilis* is an important component of the pigeon crop flora. *J Appl Microbiol* **91**, 488–491.
- Baele, M., Van den Bulck, K., Decostere, A., Vandamme, P., Hänninen, M. L., Ducatelle, R. & Haesebrouck, F. (2004). Multiplex PCR assay for differentiation of *Helicobacter felis*, *H. bizzozeronii* and *H. salomonis*. *J Clin Microbiol* **42**, 1115–1122.
- Cattoli, G., Van Vugt, R., Zanoni, R. G., Sanguinetti, V., Chiocchetti, R., Gualtieri, M., Vandenbroucke-Grauls, C. M., Gastra, W. & Kusters, J. G. (1999). Occurrence and characterization of gastric *Helicobacter* spp. in naturally infected dogs. *Vet Microbiol* **70**, 239–250.
- Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J. R. W., Kersters, K. & Vandamme, P. (1999). Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. *Int J Syst Bacteriol* **49**, 405–413.
- Costas, M., Pot, B., Vandamme, P., Kersters, K., Owen, R. J. & Hill, L. R. (1990). Interlaboratory comparative study of the numerical analysis of one-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoretic protein patterns of *Campylobacter* strains. *Electrophoresis* **11**, 467–474.
- Dewhirst, F. E., Fox, J. G. & On, S. L. (2000). Recommended minimal standards for describing new species of the genus *Helicobacter*. *Int J Syst Evol Microbiol* **50**, 2231–2237.
- Eaton, K. A., Dewhirst, F. E., Radin, M. J., Fox, J. G., Paster, B. J., Krakowka, S. & Morgan, D. R. (1993). *Helicobacter acinonyx* sp. nov., isolated from cheetahs with gastritis. *Int J Syst Bacteriol* **43**, 99–106.
- Fox, J. G., Taylor, N. S., Edmonds, P. & Brenner, D. J. (1988). *Campylobacter pylori* subsp. *mustelae* subsp. nov., isolated from the gastric mucosa of ferrets (*Mustela putorius furo*), and an emended description of *Campylobacter pylori*. *Int J Syst Bacteriol* **38**, 367–370.
- Germani, Y., Dauga, C., Duval, P., Huerre, M., Levy, M., Pialoux, G., Sansonetti, P. & Grimont, P. A. (1997). Strategy for the detection of *Helicobacter* species by amplification of 16S rRNA genes and identification of *H. felis* in a human gastric biopsy. *Res Microbiol* **148**, 315–326.
- Gruntar, I., Mehle, J., Krt, B., Ocepek, M., Gombac, M., Cerne, M. & Pogacnik, M. (2003). New approaches in isolation of *Helicobacter* spp. from gastric mucosa in dogs. *Int J Med Microbiol* **293** (Supplement), 65.
- Hänninen, M. L., Happonen, I., Saari, S. & Jalava, K. (1996). Culture and characteristics of *Helicobacter bizzozeronii*, a new canine gastric *Helicobacter* sp. *Int J Syst Bacteriol* **46**, 160–166.
- Hänninen, M. L., Utriainen, M., Happonen, I. & Dewhirst, F. E. (2003). *Helicobacter* sp. *flexispira* 16S rDNA taxa 1, 4 and 5 and Finnish porcine *Helicobacter* isolates are members of the species *Helicobacter trogonum* (taxon 6). *Int J Syst Evol Microbiol* **53**, 425–433.
- Houf, K., On, S. L. W., Coenye, T., Mast, J., Van Hoof, J. & Vandamme, P. (2005). *Arcobacter cibarius* sp. nov., isolated from broiler carcasses. *Int J Syst Evol Microbiol* **55**, 713–717.
- Jalava, K., Kaartinen, M., Utriainen, M., Happonen, I. & Hänninen, M. L. (1997). *Helicobacter salomonis* sp. nov., a canine gastric *Helicobacter* sp. related to *Helicobacter felis* and *Helicobacter bizzozeronii*. *Int J Syst Bacteriol* **47**, 975–982.

- Jalava, K., On, S. L., Vandamme, P. A., Happonen, I., Sukura, A. & Hänninen, M. L. (1998). Isolation and identification of *Helicobacter* spp. from canine and feline gastric mucosa. *Appl Environ Microbiol* **64**, 3998–4006.
- Jalava, K., On, S. L., Harrington, C. S., Andersen, L. P., Hänninen, M. L. & Vandamme, P. A. (2001). A cultured strain of “*Helicobacter heilmannii*”, a human gastric pathogen, identified as *H. bizzozeronii*: evidence for zoonotic potential of *Helicobacter*. *Emerg Infect Dis* **7**, 1036–1038.
- Lee, A., Hazell, S. L., O'Rourke, J. & Kouprach, S. (1988). Isolation of a spiral-shaped bacterium from the cat stomach. *Infect Immun* **56**, 2843–2850.
- Lee, A., Phillips, M. W., O'Rourke, J. L. & 8 other authors (1992). *Helicobacter muridarum* sp. nov., a microaerophilic helical bacterium with a novel ultrastructure isolated from the intestinal mucosa of rodents. *Int J Syst Bacteriol* **42**, 27–36.
- On, S. L. W., Bloch, B., Holmes, B., Hoste, B. & Vandamme, P. (1995). *Campylobacter hyointestinalis* subsp. *lawsonii* subsp. nov., isolated from the porcine stomach, and an emended description of *Campylobacter hyointestinalis*. *Int J Syst Bacteriol* **45**, 767–774.
- O'Rourke, J. L., Solnick, J. V., Neilan, B. A., Seidel, K., Hayter, R., Hansen, L. M. & Lee, A. (2004). Description of ‘*Candidatus Helicobacter heilmannii*’ based on DNA sequence analysis of 16S rRNA and urease genes. *Int J Syst Evol Microbiol* **54**, 2203–2211.
- Patterson, M. M., Schrenzel, M. D., Feng, Y., Xu, S., Dewhirst, F. E., Paster, B. J., Thibodeau, S. A., Versalovic, J. & Fox, J. G. (2000). *Helicobacter aurati* sp. nov., a urease-positive *Helicobacter* species cultured from gastrointestinal tissues of Syrian hamsters. *J Clin Microbiol* **38**, 3722–3728.
- Pot, B., Devriese, L. A., Hommez, J., Miry, C., Vandemeulebroecke, K., Kersters, K. & Haesebrouck, F. (1994). Characterization and identification of *Vagococcus fluvialis* strains isolated from domestic animals. *J Appl Bacteriol* **77**, 362–369.
- Stanley, J., Linton, D., Burnens, A. P., Dewhirst, F. E., Owen, R. J., Porter, A., On, S. L. & Costas, M. (1993). *Helicobacter canis* sp. nov., a new species from dogs: an integrated study of phenotype and genotype. *J Gen Microbiol* **139**, 2495–2504.
- Utriainen, M., Jalava, K., Sukura, A. & Hanninen, M. L. (1997). Morphological diversity of cultured canine gastric *Helicobacter* spp. *Comp Immunol Microbiol Infect Dis* **20**, 285–297.
- Vandamme, P., Falsen, E., Pot, B., Hoste, B., Kersters, K. & De Ley, J. (1989). Identification of EF group 22 campylobacters from gastroenteritis cases as *Campylobacter concisus*. *J Clin Microbiol* **27**, 1775–1781.
- Vandamme, P., Pot, B., Falsen, E., Kersters, K. & De Ley, J. (1990). Intra- and interspecific relationships of veterinary campylobacters revealed by numerical analysis of electrophoretic protein profiles and DNA:DNA hybridizations. *Syst Appl Microbiol* **13**, 295–303.
- Vandamme, P., Pot, B. & Kersters, K. (1991). Differentiation of campylobacters and *Campylobacter*-like organisms by numerical analysis of one-dimensional electrophoretic protein patterns. *Syst Appl Microbiol* **14**, 57–66.
- Vandamme, P., Dewettinck, D. & Kersters, K. (1992a). Application of numerical analysis of electrophoretic protein profiles for the identification of thermophilic campylobacters. *Syst Appl Microbiol* **15**, 402–408.
- Vandamme, P., Vancanneyt, M., Pot, B. & 10 other authors (1992b). Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *Int J Syst Bacteriol* **42**, 344–356.
- Vandamme, P., Pot, B., Gillis, M., De Vos, P., Kersters, K. & Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microb Rev* **60**, 407–438.
- Vandamme, P., van Doorn, L.-J., Al Rashid, S. T., Quint, W. G. V., van der Plas, J., Chan, V. L. & On, S. L. W. (1997). *Campylobacter hyoilei* Alderton et al. 1995 and *Campylobacter coli* Véron and Chatelain 1973 are subjective synonyms. *Int J Syst Bacteriol* **47**, 1055–1060.
- Vandamme, P., Harrington, C. S., Jalava, K. & On, S. L. (2000). Misidentifying helicobacters: the *Helicobacter cinaedi* example. *J Clin Microbiol* **38**, 2261–2266.
- Van den Bulck, K., Decostere, A., Baele, M., Driessen, A., Debongnie, J. C., Burette, A., Stolte, M., Ducatelle, R. & Haesebrouck, F. (2005a). Identification of non-*Helicobacter pylori* spiral organisms in gastric samples from humans, dogs and cats. *J Clin Microbiol* **43**, 2256–2260.
- Van den Bulck, K., Decostere, A., Gruntar, I., Baele, M., Krt, B., Ducatelle, R. & Haesebrouck, F. (2005b). In vitro antimicrobial susceptibility testing of *Helicobacter felis*, *H. bizzozeronii* and *H. salomonis*. *Antimicrob Agents Chemother* **49**, 2997–3000.