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NOTE

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Epidemiological study on feline gastric *Helicobacter* spp. in Japan

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ABSTRACT. Epidemiological and pathological studies on *Helicobacter* spp. in feline stomachs in Japan were conducted using genus- and species-specific (*H. felis*, *H. bizzozeronii*, *H. heilmannii* sensu stricto [s.s.] and *H. pylori*) polymerase chain reactions (PCRs), *ureAB* gene sequencing and histopathology. PCR results showed that 28 of 56 cats were infected with *Helicobacter* spp., and *H. heilmannii* s.s. was the most prevalent species by both PCR (28/28) and *ureAB* gene sequencing (26/28). Some of the sequences showed high similarities with those from human patients with gastric diseases (99%). There were no significant differences between *Helicobacter* spp.-positive and -negative cats in the severity of chronic gastritis (P=0.69). This is the first extensive epidemiological study on feline gastric *Helicobacter* spp. in Japan.

KEY WORDS: epidemiology, feline, *Helicobacter heilmannii*, Japan, zoonosis

The genus *Helicobacter* is gram-negative, microaerophilic spiral bacteria and contains at least 40 species [1]. *Helicobacter pylori* (*H. pylori*) is the predominant species in humans and associated with various human gastric diseases [12, 17, 21]; however, it has been reported only three times in cats [2, 9, 18].

Non-*H. pylori* helicobacters (NHPH) have also been detected in the stomachs of humans and several animal species. According to previous reports, the prevalence of NHPH in cats is 41–100% [8]. The predominant NHPH in cats were *H. heilmannii* sensu stricto (s.s.), *H. felis* and *H. bizzozeronii,* whereas *H. salomonis* was less often detected. *H. baculiformis* was recently isolated from a feline stomach, and its prevalence has not yet been determined [8]. Most of these NHPH are thought to be associated with human gastric diseases including active chronic gastritis, acute gastritis, gastric ulcer and gastric low-grade MALT lymphoma [5, 8, 15, 22] and are responsible for causing a growing concern as zoonosis. In contrast, there are many conflicting reports regarding the pathogenicity of each NHPH in cats, and therefore, the pathogenic significance of these gastric NHPH in cats is controversial at present [8]. The conflicting results of the pathogenicity of each NHPH in cats may occur due to the differences in virulence between different isolates, as has also been described for *H. pylori* [6, 12]. Despite their potential importance, there are insufficient epidemiological data to estimate the prevalence of *Helicobacter* spp. in feline stomachs in Japan.

The purposes of this study were to determine the prevalence of different *Helicobacter* spp. in feline stomachs in Japan and to evaluate the associations of these species with histopathological changes.

Fifty-six cats that underwent upper gastrointestinal endoscopy for various reasons at the Veterinary Medical Center of the University of Tokyo (VMC-UT) from April 2013 to June 2016 and Japan Small Animal Medical Center from June 2015 to June 2016, without a prior prescription of the effective eradication protocols for gastric *Helicobacter* spp. for cats using the combination of proton pump inhibitor (omeprazole or lansoprazole) or famotidine and two of the following: amoxicillin, metronidazole or clarithromycin [3, 7], were enrolled in this study (Table 1). Informed consent was obtained from all cat owners, and the study

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Table 1. Characteristics of cats included in this study (n=56)

Gender	
Female	24 (21 spayed)
Male	32 (30 castrated)
Ages (years)	1.6 to 15.0 (median: 9.3)
Body weight (kg)	1.8 to 8.8 (median: 4.1)
Breeds	Mixed breed (n=36), Russian Blue (n=5), American Shorthair (n=4), Maine Coon and Abyssinian (n=2 each) and others (n=7)
Chief complaints	Vomiting (n=37), anorexia (n=16), diarrhea or bloody stool (n=15), weight loss (n=10) and others (n=6)

protocol was approved by the animal care committee of VMC-UT. Gastric biopsy samples were obtained under anesthesia from the antrum, corpus and fundus, in this order, using an endoscope designed for animals (the Olympus VQ-8143B; Olympus Medical Systems Corp., Tokyo, Japan) and biopsy forceps (FB-54Q-1; Olympus Medical Systems Corp.). The biopsy forceps were washed intensively with water and 70% ethanol in between each collection from the targeted gastric regions. The samples obtained were subjected to polymerase chain reaction (PCR) and histopathology.

DNA was extracted from specimens using a QIAamp DNA Mini Kit (Qiagen, Santa Clarita, CA, U.S.A.). Genus-specific PCR was performed, followed by species-specific (*H. felis*, *H. bizzozeronii*, *H. heilmannii* s.s. and *H. pylori*) PCR and a sequencing analysis of the partial *ureAB* gene to investigate *Helicobacter* at the strain level. Primers and amplification parameters for each specific reaction are shown in Tables S1 and S2, respectively.

The PCR products of the partial *ureAB* gene from *Helicobacter* spp.-positive samples were purified and cloned using the Wizard PCR Preps DNA Purification System (Promega Corp., Madison, WI, U.S.A.), 10× A-attachment mix (Toyobo Co., Ltd., Osaka, Japan) and pGEM-T Easy vector (Promega), followed by sequencing of plasmid DNA from eight clones per sample using BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA, U.S.A.). Homology sequences matches were searched in the NCBI/GenBank database using the BLAST search tool.

A phylogenetic tree was constructed using the neighbor-joining method from sequences of clinical strains obtained in this study, the sequences of *Helicobacter* spp. in NCBI/GenBank most closely related to each clinical strain and the partial *ureAB* genes from urease-positive reference *Helicobacter* spp. [19, 20].

Histopathological evaluations were performed by two pathologists (K. U. and J. C.). The gastric biopsies were evaluated for evidence of gastritis according to the World Small Animal Veterinary Association criteria, and changes were graded as normal, mild, moderate or severe [4].

Fisher's exact test was used to determine the association between the *Helicobacter* status (positive or negative) and category of gastritis (normal to mild or moderate to severe). Statistical analyses were performed using JMP Pro 11 (SAS Institute, Cary, NC, U.S.A.), with the level of significance set at P=0.05.

PCR results showed that 28 of 56 cats were infected with *Helicobacter* spp. (50%): *H. heilmannii* s.s. (25/28), *H. bizzozeronii* (7/28), *H. felis* (7/28) and *H. pylori* (0/28). Three of the genus-positive samples could not be amplified by any of the species-specific primers. The prevalence of *Helicobacter* spp. in this study falls within the range previously reported in cats outside of Japan (41–100%) [8]. The predominant *Helicobacter* species in the present study, as determined by PCR, was *H. heilmannii* s.s. (89%), a finding similar to those of epidemiological studies in Switzerland (86%) and Korea (86%) [10, 16], whereas the infection rate of *H. felis* in the present study (25%) was higher than those reported in Switzerland (0.0%) and Korea (9.5%). The lack of *H. pylori* infections found in the present study was the same as that found in these other countries. The prevalence of *H. bizzozeronii* was not determined in the previous studies. Correlations between the *Helicobacter* spp. infection rate and age and sex were not statistically significant. The relationship between the *Helicobacter* spp. infection rate and type of breed could not be investigated owing to the small number of each pure breed included in the present study. The detection rate of *Helicobacter* spp. was the highest in the gastric fundus (50%), followed by the corpus (43%) and antrum (21%), which is identical to the tendency observed in dogs in Japan [11]. There were no differences between each of the *Helicobacter* species regarding the tendency of the detection rate in the three gastric regions.

To investigate *Helicobacter* at the strain level, partial *ureAB* genes from 27 of the 28 genus-positive samples were sequenced, and 24 unique sequences were obtained (GenBank/EMBL/DDBJ accession numbers are shown in Fig. 1). No amplification was observed in one case. Homology matches identified using the BLAST program revealed that 18 strains from 26 cases were most closely related to *H. heilmannii* s.s. (AB778507, L25079, AB462258 or HM625826), with sequence similarities of 91–99%; two strains from two cases were most closely related to *H. bizzozeronii* (FR871757, 96–98%); and four strains from five cases were most closely related to *H. felis* (FQ670179, 98–99%) (Table 2). Of these sequences, nine strains showed high similarities (99%) with those of *H. heilmannii* s.s. strains (L25079, AB778507 and AB462258) that were obtained from human patients with gastric diseases, suggesting that these strains may contribute to zoonosis [13, 14, 20]. Another nine strains were most closely related (about 90% similarity) to known *H. heilmannii* s.s. strains, suggesting that there are novel subspecies of *H. heilmannii* s.s. in Japan.

Mild to severe chronic gastritis were the most frequently observed conditions in both *Helicobacter* spp.-positive and -negative cases (Table 3). The proportion of cats with moderate to severe chronic gastritis did not differ between the groups (P=0.69). *H. heilmannii* s.s. strains which were most closely related to those isolated from human patients with gastric diseases with sequence similarities of 99% were detected in 22 cats of the 28 *Helicobacter* spp.-positive cases, but there was no significant difference in

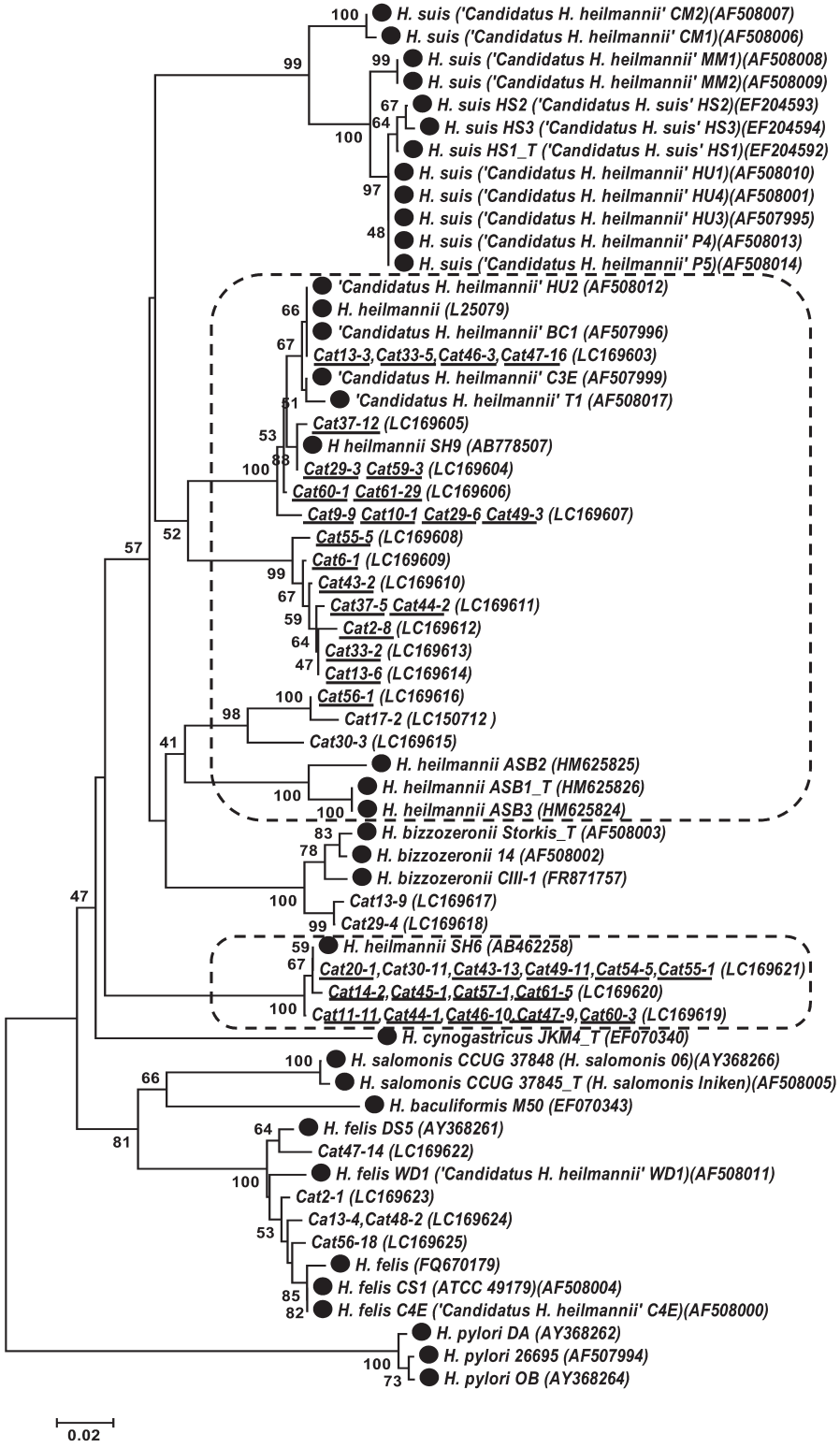

Fig. 1. A phylogenetic tree constructed based on genetic distances and from the partial *ureAB* gene sequences amplified from 27 *Helicobacter*-positive cats and other urease-positive *Helicobacter* species. All strain names are accompanied by GenBank/EMBL/DDBJ accession numbers. Strains with a black circle (●) at the beginning of their names are reference strains, whereas strains without a black circle are clinical strains obtained in this study. Strains with "T" at the end of their names and in front of accession numbers are type strains for their species. Clinical strain names include each cat's individual identification number, and the numbers after hyphens indicate clones. The 24 strains in the box with a dotted line are thought to constitute *H. heilmannii* s.s. clusters. The clinical strains with underlines in the box with a dotted line indicate strains that were obtained from samples found positive for *H. heilmannii* s.s. by species-specific PCR. Bootstrap values (for branches present in more than 40% of 1,000 resamplings of the data) are indicated. *H. pylori strains* DA, 26695 and OB were used as an outgroup. Evolutionary analyses were conducted in MEGA 6.0.6.

Table 2. Sequence similarities of the partial *ureAB* gene from 24 strains detected in the stomachs of 27 *Helicobacter*-positive cats

Number of strains (cases detected)	Most closely related species		Similarity	
	Species	Gene accession no.	Within the specie (%)	Among the clinical strains (%)
18 (26)	*H. heilmannii* s.s.	AB778507 L25079 AB462258 HM625826	91–99	90–99
2 (2)	*H. bizzozeronii*	FR871757	96–98	97
4 (5)	*H. felis*	FQ670179	98–99	98–99

Table 3. Histopathological diagnoses of feline stomachs (n=56)

Histopathological diagnosis		Not infected with *Helicobacter* spp. (n=28) / (%)	Infected with *Helicobacter* spp. (n=28) / (%)
Normal		1 (3.6)	0 (0.0)
Chronic gastritis	Mild	19 (68)	15 (54)
	Moderate	2 (7.1)	2 (7.1)
	Severe	0 (0.0)	2 (7.1)
Atrophic gastritis		1 (3.6)	0 (0.0)
Gastric adenocarcinoma		0 (0.0)	1 (3.6)
Lymphoma	Low-grade	1 (3.6)	2 (7.1)
	High-grade	2 (7.1)	5 (18)
	LGL[a)]	2 (7.1)	0 (0.0)
Amyloidosis		0 (0.0)	1 (3.6)

a) Large granular lymphocytic.

the severity of gastritis between these strains-positive and *Helicobacter* spp.-negative groups (P=0.67). A comparison of gastric histopathological diagnoses between cases infected with different NHPH could not be performed, because *H. heilmannii* s.s. was detected in almost all the *Helicobacter*-positive cases (26/28), as determined by *ureAB* gene sequencing. Gastric lymphomas (low-grade or high-grade) were seen in both *Helicobacter* spp.-positive cats (25%) and -negative (11%) cats, while gastric carcinoma and amyloidosis were seen only in infected cats, and atrophic gastritis and large granular lymphocytic lymphoma were seen only in uninfected cats. Gastric adenocarcinoma was seen in a cat infected with *Helicobacter* species which was most closely related to *H. heilmannii* s.s. SH6 (AB462258, with similarity of 99%), which have been detected in a human patient with gastritis, and amyloidosis was observed in a cat infected with one which was similar to *H. felis* (FQ670179; 99%) by the sequence analysis of the partial *ureAB* gene.

There were some limitations to our study: all cases included in this study showed some signs of gastrointestinal disease, we utilized only two referral veterinary centers in Tokyo and Saitama, and some cases had been administered antibiotics prior to this study. These factors might have affected our determinations of the *Helicobacter* spp. infection rate and the proportion of each NHPH found in this study. In addition, the investigation of each NHPH pathogenicity was insufficient, because a comparison of gastric histopathological diagnoses between cases infected with different NHPH could not be performed in this study because *H. heilmannii* s.s. was detected in almost all (26/28) of the *Helicobacter*-positive cases, as determined by partial *ureAB* sequencing. To properly investigate the pathogenicity of each NHPH, *in vitro* cultivation of each NHPH and experimental infection of feline gastric cell lines or cats will be necessary.

In summary, this is the first extensive epidemiological study on feline *Helicobacter* spp. in Japan. *H. heilmannii* s.s. was the predominant *Helicobacter* species in feline stomachs, and some of the strains represented a possible zoonosis. Further studies, including *in vitro* cultivation of each NHPH, will be necessary to properly investigate the pathogenicity of each NHPH.

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