Turk J Vet Anim Sci
26 (2002) 345-351

Research Article

The Prevalence, Colonization Sites and Pathological Effects of Gastric Helicobacters in Dogs

K. Serdar DİKER
Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, 06110, Ankara-TURKEY
Rıfkı HAZIROĞLU
Department of Pathology, Faculty of Veterinary Medicine, Ankara University, 06110, Ankara-TURKEY
Mehmet AKAN, Serap ÇELİK
Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, 06110, Ankara-TURKEY
Nalan KABAKÇI
Department of Pathology, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale-TURKEY

Received: 28.04.2001

Abstract: The prevalence and colonization sites of *Helicobacter* spp. in the stomachs of dogs, and their association with gastric pathology were investigated. Scraping cytology, culture, urease test and histology were used to detect helicobacters in the stomachs of necropsied dogs. Gastric *Helicobacter* spp. were detected in 103 (84.4%) of 122 dogs from 1 month to 14 years of age. The uncultured spiral organisms seen in the most of stomachs were designated as *H. heilmannii*. Microscopical examination of stained mucosal scrapings was found to be superior for the diagnosis of gastric *helicobacter*s. Six (4.9%) spiral organisms were isolated from 122 stomachs and all were identified as *H. felis*. *Helicobacter* spp. were detected in the fundus, corpus and antrum of 103, 101 and 53 dogs, respectively. Organisms were denser in the fundus and corpus than in the antrum. Histological changes comparable to mild chronic gastritis or chronic active gastritis were found in 56.3% of *Helicobacter*-positive dogs and 47.4% of *Helicobacter*-negative dogs. *H. felis* was found only in dogs with chronic active gastritis.

Key Words: *Helicobacter heilmannii*, *Helicobacter felis*, dog, stomach, gastritis

Helicobacter Türlerinin Köpek Midelerindeki Sıklığı, Yerleşim Bölgeleri ve Patolojik Etkileri

Özet: *Helicobacter* türlerinin köpek midelerindeki sıklığı ve yerleşme bölgeleri ile gastric patoloji ile ilişkileri incelendi. *Helicobacter* türlerinin midedeki tesbiti için, kazıntı sitolojisi, kültür, üreaz testi ve histolojik muayene kullanıldı. Bir ay-14 yaş arasındaki 122 köpeğin 103'ünde (%84.4) gastrik *Helicobacter* türleri saptandı. Midelerin çoğunda görülen ve üretilemeyen spiral organizmalar *H. heilmannii* olarak adlandırıldı. Gastrik helikobakterlerin teşhisinde en uygun yolun mukozal kazıntıların mikroskopik muayenesi olduğu anlaşıldı. Mide örneklerinin 6'sından (%4.9) izole edilen spiral organizmalar H.felis olarak ayrıldı. *Helicobacter* türleri 103 fundus, 101 korpus ve 53 antrum örneğinde saptandı. Organizmalar, fundus ve korpusta, antruma göre daha yoğun olarak bulundu. Hafif düzeyde kronik gastritis veya kronik aktif gastritise uyumlu histopatolojik bulgular *Helicobacter*-pozitif köpeklerin %56.3'ünde, *Helicobacter*-negatif köpeklerin %47.4'ünde belirlendi. H.felis, sadece kronik aktif gastritli köpeklerde bulundu.

Anahtar Sözcükler: *Helicobacter heilmannii*, *Helicobacter felis*, köpek, mide, gastritis

Introduction

Interest in gastric spiral bacteria has increased following the implication of *Helicobacter pylori* in gastritis and peptic ulcer in man (1). Since then, 28 *Helicobacter* species have been described in the stomachs and intestines of animals and humans (http://www.ncbi.nlm.nih.gov/Taxonomy). Four *Helicobacter* species have been isolated from the stomach of dogs: *H. felis*, *H. bizzozeronii*, *H. salomonis* and *H. rappini* (2-5). In addition to these validly described species, uncultured helicobacters also colonize the gastric mucosa of dogs (6-8). These organisms have been tentatively named "*Gastrospirillum hominis*" in their original description (9). Following 16S rDNA sequence analysis of similar uncultured bacteria isolated from the human stomach, these organisms have been indicated as true helicobacters and assigned as *H. heilmannii* (10). Canine gastric helicobacters including *H. felis*, *H. bizzozeronii*, *H. salomonis* and *H. heilmannii* (or *G. hominis*) are Gram-negative, urease-producing spiral organisms and were

differentiated, as a group, from other *Helicobacter* sp. by 23S rRNA gene polymorphism (11,12). Gastric helicobacters have been reported to be very common among dog populations; they have been found in asymptomatic dogs as much as in dogs with gastric lesions (13-18). In most reports, the uncultured organism *H. heilmannii* has been shown to be the single or dominant species colonizing the gastric mucosa of dogs. However, following the successful isolation of *H. bizzozeronii*, this organism has been implicated as another inhabitant of canine gastric mucosa (5,19). The high prevalence of gastric helicobacters in dogs may have several implications. First, dogs may be a source of infection for human beings; *H. heilmannii* has been isolated from humans with gastric pathology (20,21). Second, the clinical significance of gastric helicobacters in dogs is of interest from a veterinary viewpoint; they may cause gastric and/or related diseases. Finally, gastric helicobacters of dogs may be used as an animal model for human disease.

The aims of this study were to determine the prevalence and colonization sites of helicobacters in the stomachs of dogs, and their association with gastric pathology.

Materials and Methods

Specimens for the examination of helicobacters were obtained from 122 dogs submitted to the Pathology Department, Faculty of Veterinary Medicine of Ankara University, between 1991 and 1997. The dogs were one week to 14 years old and had died naturally or had been euthanatized for various illnesses. Only dogs handled within 18 h of death were included in the study. At necropsy, stomachs were removed and opened along the greater curvature and samples were collected from the fundus (fundus plus cardia), corpus, and antrum (antrum plus pylorus).

Mucosal scrapings from all sample sites were stained with Giemsa, Gram and carbol fuchsin for the examination of spiral organisms by light microscopy. For scanning electron microscopy (SEM), samples from six *H. heilmannii*-infected dogs were fixed, dehydrated, coated with gold and examined by SEM (TopCon Abt60). For transmission electron microscopy (TEM), samples from three *H. felis*-infected dogs and three *H. heilmannii*-infected dogs were fixed in gluteraldehyde and processed routinely. Thin sections were examined with a Zeiss EM9S.

To detect the preformed urease activity in the stomachs of dogs, small pieces from the fundus, corpus and antrum were placed in rapid urease test medium (22) with phenol red indicator at room temperature. The test was scored as positive if the indicator turned pink or red within 3 h.

Scrapings from the fundus, corpus and antrum of all dogs were inoculated onto the following media: Skirrow selective medium with 5% lysed horse blood, brucella agar with 5% lysed horse blood, thioglycolate agar with 5% defibrinated sheep blood and chocolate agar. All media were incubated at 37ºC in a microaerobic atmosphere (5% O_2, 10% CO_2 and 85% H_2) for one week. Cultured organisms were identified according to criteria described previously (3-5).

Only stomachs in which autolysis was absent or minimal were handled for histological examination. Fundus, corpus and antrum samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were processed with conventional methods and stained with haematoxylin and eosin. Histological changes were classified according to the criteria described by Happonen et al. (15).

Results

Helicobacter species were detected in the stomachs of 103 (84.4%) dogs by direct microscopical examination of stained mucosal scrapings. Rapid urease test was positive in 88 (72.1%) stomachs and spiral organisms were seen in the histological sections of 80 (65.6%) stomachs. Six (4.9%) spiral organisms were isolated from 122 stomachs (all from positive samples in direct microscopical examination) and identified as *H. felis* by their phenotypic characteristics. All *H. felis* strains could be isolated only on Skirrow selective medium.

On microscopical examination of mucosal scrapings, organisms were found to be single or in groups. All organisms were Gram-negative, long and tightly coiled spirals (Fig. 1). On TEM examination of specimens from three *H. felis*-infected dogs, organisms were seen as 4-8 µm long spirals with 6-8 turns (Fig. 2). *H. felis* was further characterized by periplasmic fibrils and loosely coiled spirals. Uncultured organisms with more tightly

Figure 1. Helicobacters in gastric mucosal scraping of a dog. Carbol fuchsin (X1000).

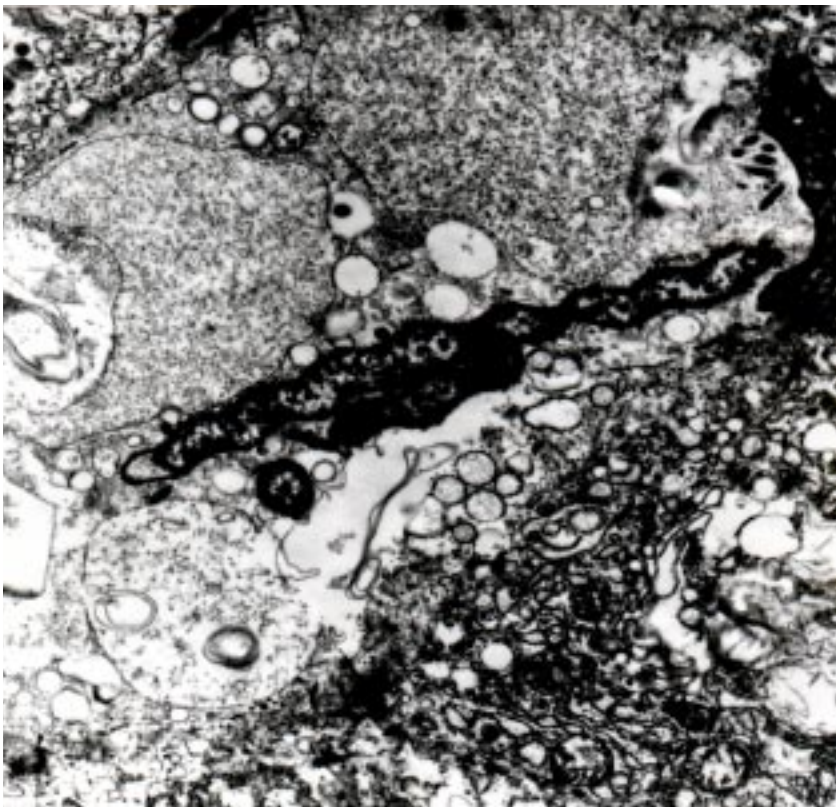

Figure 2. *H. felis* in the gastric tissue of a dog. Note the periplasmic fibrils and loosely coiled spirals. TEM (X4500).

coiled spirals had no periplasmic fibrils on TEM examination of samples from other dogs (Fig. 3). Based on these morphological characteristics, the uncultured organisms seen in the most samples were identified as *H. heilmannii*. *H. heilmannii* was detected in all SEM samples in high numbers (Fig. 4).

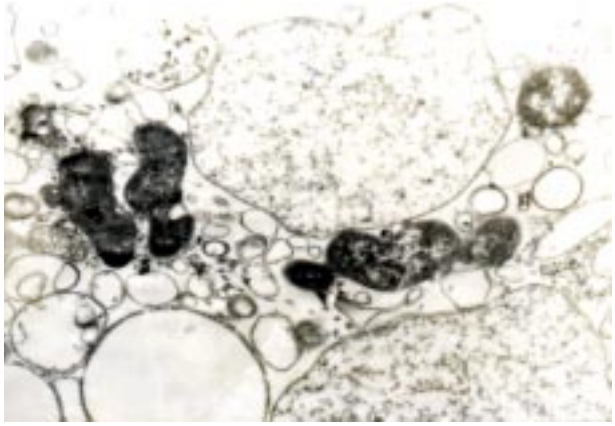

Figure 3. *H. heilmannii* in the gastric tissue of a dog. Note the tightly coiled spirals without periplasmic fibrils. TEM (X7500).

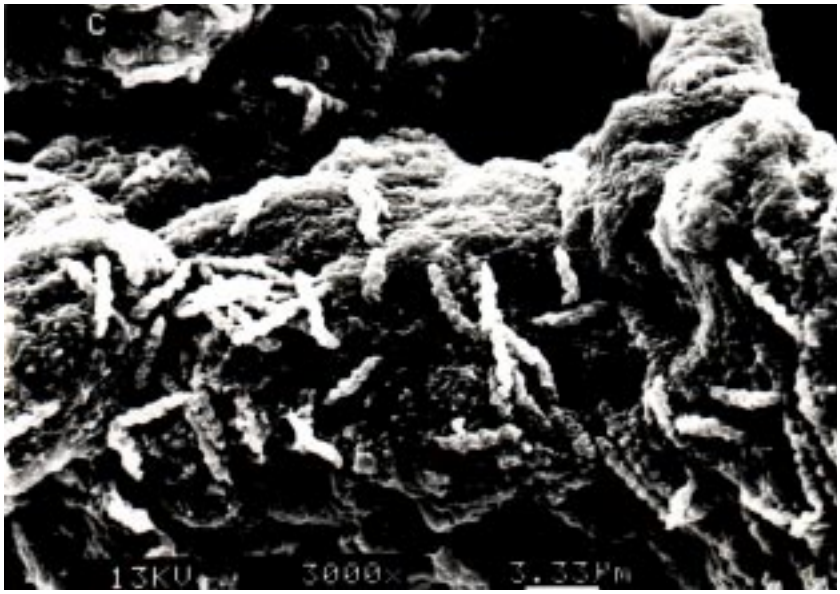

Figure 4. High numbers of *H. heilmannii* on the mucosal surface of the stomach. SEM (X3000).

One of 6 (16.7%) puppies younger than one month of age, 9 of 16 (56.3%) dogs between two months and one year of age, and 93 of 100 (93.0%) dogs older than one year of age were found to carry helicobacters in their stomachs. *H. felis* was isolated only from dogs older than one year of age.

Helicobacters were detected in the fundus of all (100%) 103 positive dogs, in the corpus of 101 (98.1%) dogs, and in the antrum of 53 (51.5%) dogs. The organisms were also denser in the fundus and corpus than in the antrum, as detected by microscopical examination and urease test.

Histological changes comparable to mild chronic gastritis (characterized by lymphocytes, plasma cells and lymphocyte aggregates) were seen in 67 dogs, of which 34 also had mild chronic active gastritis (characterized additionally by neutrophils). No difference was found in the appearance of chronic or active gastritis among different regions of the stomachs. Gastritis was detected in 58 (56.3%) of 103 *Helicobacter*-positive dogs and 9 (47.4%) of 19 *Helicobacter*-negative dogs. Helicobacters were observed in 58 (86.6%) of 67 dogs with gastritis, and 45 (81.8%) of 55 dogs without gastric lesions. Almost all of the gastritis cases were detected in dogs

older than one year of age. In histological sections, gastric spiral organisms were seen in the mucus adjacent to surface epithelium, glandular lamina adjacent to parietal cells and gastric pits. The culturable organism *H. felis* was found in dogs with chronic active gastritis, but not in dogs with normal gastric histology.

Discussion

The uncultured spiral organisms seen in the stomachs of most dogs were structurally different from those isolated from our dogs and identified as *H. felis*, because they had no periplasmic fibrils and more tightly coiled spirals. We designated the uncultured organisms as *H. heilmannii*, which was proposed originally for human-derived strains (9). Whether this name for dog-originated strains will find favour remains to be seen, but some sort of designation is needed, because other reports of H. eilmannii appear from around the world and many new members join the *Helicobacter* group.

The colonization of the dog stomach by helicobacters seemed to be a common event and appeared to increase with age, in this study. This is a common finding of similar research; gastric *Helicobacter* spp. have been identified in 67% to 100% of pet dogs and laboratory and shelter dogs (3,6,8,13,14,16,17). Information regarding the frequency of different *Helicobacter* spp. in the dog stomach is scarce. In a recent study reporting the highest isolation rates, helicobacters were cultured from 51% of dogs and the distributions of *H. bizzozeronii*, *H. felis*, *H. salomonis* and *H. rappini* were found to be 55.6%, 22.2%, 22.2% and 4.4%, respectively (5). Among these species, *H. bizzozeronii*, described in 1996 (3), and *H. salomonis*, described in 1997 (4), were not found in our study. Since our study was conducted between 1991 and 1997, we were unable to employ the strict culture measures necessary for the isolation of these species. In any case, uncultured *H. heilmannii* has also been reported to be the single or dominant organism in dog stomachs in other studies (8,13,14,16,17). Colonization of helicobacters in the dog stomach appeared to be similar to the colonization of *H. pylori* in the human stomach (23), since the prevalence of organisms increased with age. The in vitro survival of gastric helicobacters in the stomach content of dogs for at least 15 days as reported previously (24) may allow the transmission of organisms from dog to dog easily.

Among the diagnostic methods used in this study, direct examination of stained mucosal scrapings was found to be the most sensitive method for detecting gastric helicobacters. This method has also determined the highest rate of gastric helicobacters in other studies (17,25). The rapid urease test detecting preformed urease produced by helicobacters in gastric tissue was found to be a less sensitive method. Urease activity of gastric tissue appeared to be associated with the density of helicobacters, since urease negative samples usually contained a small number of organisms.

The fundus and corpus were found to be primary colonization sites of helicobacters in the stomachs of dogs. All diagnostic methods used in this study confirmed this suggestion. Although colonization of helicobacters in all parts of the stomach is possible, in a study on topographical mapping of helicobacters, organisms have been detected in the fundus and corpus of all stomachs (15).

In this study, histological changes comparable to mild chronic gastritis were seen in 67 (54.9%) of 122 dogs. Gastritis was detected only in two dogs younger than one year of age. Vomiting was documented as one of the clinical signs in 24 dogs carrying helicobacters. This finding was not included in the final evaluation, since reliable case histories of other dogs could not be obtained. When comparing the prevalence of helicobacters in normal dogs and dogs with gastritis, a direct association between helicobacters and gastritis could not be established as in other studies (13,14,16,17). The prevalence of helicobacters in normal dogs was as high as in dogs with gastritis. However, the high prevalence of gastric helicobacters in normal dogs should not exclude the potential of these organisms to produce gastritis. As an analogy, *H. pylori* is the inevitable aetiologic agent of human gastritis, but most individuals in certain populations carry the organism without any sign of gastric pathology (23). This phenomenon is partly related to the pathogenic phenotypes of *H. pylori*. Similar pathogenic phenotypes may exist among canine gastric helicobacters, and this may explain why not all helicobacters caused clinical gastritis in dogs. Similarly, a constant pathogenic effect of *H. felis* in beagles, but not of *H. bizzozeronii*, has been determined histologically (26). In our study, the presence of *H. felis* in only dogs with relatively severe active gastritis, but not in normal dogs, confirmed this finding. Since knowledge on the

pathogenic mechanisms of canine gastric helicobacters and the histological criteria for canine gastritis is premature, further studies are needed on this subject.

The zoonotic transmission of gastric helicobacters from dogs to humans has been suggested as a likely possibility and pet ownership is considered a risk factor (23,27). *H. heilmannii* has been identified in 0.3 to 8% of humans with gastric helicobacteriosis and this prevalence may be underestimated due to difficulties in the diagnosis of this organism (20,21). In any case, the high prevalence of gastric helicobacters in dogs and documented cases of human *H. heilmannii* infections increase the zoonotic transmission of organisms to humans in close contact with dogs.

References

1. Marshall, B.J., Warren, J.R.: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet, 1984; i: 1311.
2. Haziroglu, R., Diker, K.S., Guvenc, T., Kul, O.: Canine gastritis associated with *Helicobacter felis*. Dtsch. Tierarztl. Wschr., 1995; 102: 474-476.
3. Hanninen, M.L., Happonen, I., Saari, S., Jalava, K.: Culture and characteristics of *Helicobacter* bizzozeronii, a new canine gastric *Helicobacter* sp.. Int. J. Syst. Bacteriol., 1996; 46: 160-166.
4. Jalava, K., Kaartinen, M., Utriainen, M., Happonen, I., Hanninen, M.L.: *Helicobacter* salomonis sp. nov., a canine gastric *Helicobacter* sp. related to *Helicobacter felis* and *Helicobacter* bizzozeronii. Int. J. Syst. Bacteriol., 1997; 47: 975-982.
5. Jalava, K., On, S.L.W., Vandamme, P.A.R., Happonen, I., Sukura, A., Hanninen, M.L.: Isolation and identification of *Helicobacter* spp. from canine and feline gastric mucosa. Appl. Environ. Microbiol., 1998; 3998-4006.
6. Henry, G.A., Long, P.H., Burns, J.L., Charbonneau, D.L.: Gastric spirillosis in beagles. Am. J. Vet. Res., 48: 831-836.
7. Diker, K.S., Hascelik, K., Diker, S.: Development of a technique for detecting antimicrobial susceptibility of unculturable gastric spiral bacterium *Gastrospirillum hominis*. J. Antimicrob. Chemother., 1994; 33: 867-870.
8. Geyer, C., Colbatzky, F., Lechner, J., Hermanns, W.: Occurrence of spiral shaped bacteria in gastric biopsies of dogs and cats. Vet. Rec., 1993; 133: 18-19.
9. McNulty, C.A.M., Dent, J.C., Curry, A., Uff, J.S., Ford, G.A., Gear, M.W.L., Wilkinson, S.P.: New spiral bacterium in the gastric mucosa. J. Clin. Pathol., 1989; 42: 585-591.
10. Solnick, J.V., O'Rourke, J., Lee, A., Paster, B.J., Dewhirst, F.E., Tompkins, L.S.: An uncultured gastric spiral organism is a newly identified *Helicobacter* in humans. J. Infect. Dis., 1993; 168: 379-385.
11. Jalava, K., Hielm, S., Hirvi, U., Hanninen, M.L.: Evaluation of a molecular identification scheme based on 23S rRNA gene polymorphisms for differentiating canine and feline gastric *Helicobacter* spp.. Lett. Appl. Microbiol., 1999; 28: 269-274.
12. Strauss-Ayali, D., Simpson, K.W.: Gastric *Helicobacter* infection in dogs. Vet. Clin. N. Am. Small. Anim. Pract., 1999; 29: 397-414.
13. Hermanns, W., Kregel, K., Breuer, W., Lechner, J.: *Helicobacter*-like organisms: histopathological examination of gastric biopsies from dogs and cats. J. Comp. Pathol., 1995; 112: 307-318.
14. Eaton, K.A., Dewhirst, F.E., Paster, B.J., Tzellas, N., Coleman, B.E., Paola, J., Sherding, R.: Prevalence and varieties of *Helicobacter* species in dogs from random sources and pet dogs: animal and public health implications. J. Clin. Microbiol., 1996; 34: 3165-3170.
15. Happonen, I., Saari, S., Castren, L., Tyni, O., Hanninen, M.L., Westermarck, E.: Occurrence and topographical mapping of gastric *Helicobacter*-like organisms and their association with histological changes in apparently healthy dogs and cats. J. Vet. Med. A, 1996; 43: 305-315.
16. Happonen, I., Linden, J., Saari, S., Karjalaienen, M., Hanninen, M.L., Jalava, K., Westermarck, E.: Detection and effects of helicobacters in healthy dogs and dogs with signs of gastritis. J. Am. Vet. Med. Assoc., 1998; 213: 1767-1774.
17. Yamasaki, K., Suematsu, H., Takahashi, T.: Comparison of gastric lesions in dogs and cats with and without gastric spiral organisms. J. Am. Vet. Med. Assoc., 1998; 212: 529-533.
18. Strauss-Ayali, D., Simpson, K.W., Schein, A.H., McDonough, P.L., Jacobson, R.H., Vanentine, B.A., Peacock, J.: Serological discrimination of dogs infected with gastric *Helicobacter* spp. and uninfected dogs. J. Clin. Microbiol., 1999; 37: 1280-1287.
19. Hanninen, M.L., Jalava, K., Saari, S., Happonen, I., Westermarck, E.: Culture of "Gastrospirrilum" from gastric biopsies of dogs. Eur. J. Clin. Microbiol. Infect. Dis., 1995; 14:145-146.
20. Heilmann, K.L., Borchard, F.: Gastritis due to spiral shaped bacteria other than *Helicobacter pylori*: clinical, histological and ultrastructural findings. Gut, 1991; 32: 137-140.
21. Andersen, L.P., Boye, K., Blom, J., Holck, S., Norgaard, A., Elsborg, L.: Characterization of a culturable "*Gastrospirillum hominis*" (*Helicobacter heilmannii*) strain isolated from human gastric mucosa. J. Clin. Microbiol., 1999; 37: 1069-1076.
22. Hazell, S.L., Borody, T.J., Gal, E., Lee, A.: Campylobacter pyloridis gastritis I: detection of urease as a marker of bacterial colonization and gastritis. Am. J. Gastroenterol., 1987; 82: 292-296.
23. Dubois, A.: Spiral bacteria in the human stomach: the gastric helicobacters. Emerg. Infect. Dis., 1995; 1: 1-9.

24. Diker, K.S., Lee, A.: In vitro survival of gastric spiral bacterium from dogs. Microb. Ecol. Hlth. Dis., 1991; 4: S200.

25. Happonen, I., Saari, S., Castren, L., Tyni, O., Hanninen, M.L., Westermarck, E.: Comparison of diagnostic methods for detecting gastric *Helicobacter*-like organisms in dogs and cats. J. Comp. Pathol., 1996; 115: 117-127.

26. Peyrol, S., Lecoindre, P., Berger, I., Deleforge, J., Chevallier, M.: Differential pathogenic effect of two *Helicobacter*-like organisms in dog gastric mucosa. J. Submicrosc. Cytol. Pathol., 1998; 30:425-433.

27. Svec, A., Kordas, P., Pavlis, Z., Novotny, J.: High prevalence of *Helicobacter heilmannii*-associated gastritis in a small, predominantly rural area: further evidence in support of a zoonosis? Scand. J. Gastroenterol., 2000; 35:925-928