**FULL PAPER** *Public Health*

# Determination of the Prevalence of *Helicobacter heilmannii*-Like Organisms Type 2 (HHLO-2) Infection in Humans and Dogs Using Non-Invasive Genus/Species-Specific PCR in Korea

Tae-Ho CHUNG[1)]**, Hee-Dong KIM[2)]**, Young-Sun LEE[3)] and Cheol-Yong HWANG[2)]*

[1)]*Department of Companion Animal and Animal Resources Science, Joongbu University, 1 Daehak-ro, Chubu-myeon, Geumsan-gun, Chungnam 312–702 South Korea*
[2)]*College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151–742 South Korea*
[3)]*Department of Statistics, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151–742 South Korea*

(Received 6 May 2013/Accepted 6 September 2013/Published online in J-STAGE 20 September 2013)

ABSTRACT. *Helicobacter* spp. may have multiple routes of transmission. It is unclear, however, whether the agent is zoonotic and therefore transmitted from an animal reservoir, including dogs. The aim of this population-based study was to assess the relationship between pet ownership or frequent exposure to dogs and *Helicobacter* spp. infection, especially focusing on HHLO-2 (*Helicobacter heilmannii*-like organisms type 2) in saliva and feces samples in Korea, using non-invasive genus/species-specific PCR. One hundred twenty-four eligible human subjects and 39 dogs participated in this study. Relativity of contact with dogs and *Helicobacter* spp. infection diagnosed by genus-specific PCR showed a statistically significant result ($P<0.01$), but in the relativity analyses between contact with dogs and *H. pylori, H. felis* and *H. bizzozeronii* infections diagnosed using species-specific PCR, only *Helicobacter felis* showed a statistically significant result. Although *H. pylori* infection showed a statistically significant relativity, no statistically significant association was found between veterinarian subjects and *Helicobacter.* spp., *H. felis* and *H. bizzozeronii* infections. On performing risk factor analyses of HHLO-2 infection by transmission, using matching species, between HHLO-2-positive dog owners and HHLO-2-positive dogs, *Helicobacter felis* infection showed an extremely significant relativity ($P<0.0001$), and *Helicobacter bizzozeronii* may also be a possible significant risk factor ($P<0.01$). These results suggest that HHLO-2 infection might be a zoonotic infection, because continuous contact with dogs was proved to be correlated with human *H. felis* and *H. bizzozeronii* infections in this study.

KEY WORDS: *Helicobacter bizzozeronii*, *Helicobacter felis*, *Helicobacter pylori*, HHLO-2 (*Helicobacter heilmannii*-like organisms type 2), PCR.

doi: 10.1292/jvms.13-0223; *J. Vet. Med. Sci.* 76(1): 73–79, 2014

*Helicobacter* spp. are gram-negative and spiral-shaped bacteria. Till now, almost 24 *Helicobacter* spp. have been reported, and most are suspected or proven gastric or hepatic pathogens [1]. Spiral organisms, such as *Helicobacter* spp., have been observed in domestic feline and canine gastric tissues since the last 100 years. These gastricbacteria have received special attention, and it seems that the stomachs of many types of domestic or companion animal species harbor their own *Helicobacter* spp. with long-term evolution. The *Helicobacter* spp. infection rate is 40 to 80% throughout the world in humans, and the most common *Helicobacter* spp. infection in humans in half of the world's population is *H. pylori* [2]. Recently, *H.pylori* was identified in domestic cats obtained from a commercial breeder, which suggests that pets may be a potentialsource of *H. pylori* infection [3].

General diagnostic methods for *Helicobacter* infection are divided into invasive and non-invasive methods [4]. ELISA has been developed and used for making the diagnosis of Helicobacter species, and direct observation of *Helicobacter* spp. organisms in biopsied specimens usually requires the use of special stains, e.g. Giemsa, WSS, Genta and alcian yellow-toluidine blue stains. Typically, *H.pylori* is not visualized by H&E staining [5]. Culture with biopsy of the historically affected region is the gold standard in invasive measures; however, this method is less sensitive in most *Helicobacter* species. In non-invasive methods, the PCR assay has a higher sensitivity and specificity than the other non-invasive methods, such as serological tests, and nested PCR analysis for *Helicobacter* spp. increases both the sensitivity and specificity than a single PCR analysis [6].

However, epidemiologic data of other *Helicobacter* spp., such as *H. felis* and *H. bizzozeronii,* are lacking to support the animal and public health implications. *Helicobacter* spp. have been detected continuously in humans, mainly *H. pylori,* and *Helicobacter* spp., such as *H.felis* and *H. bizzozeronii,* have been detected in pets in Korea [7]. Despite the advances in understanding the pathophysiology and virulence determinants of *H. pylori* infection, knowledge of

*CORRESPONDENCE TO: HWANG, C.-Y., College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151–742 South Korea.
e-mail: cyhwang@snu.ac.kr
**Chung, T.-H. and Kim, H.-D. contributed equally to this paper.

*H. felis* or *H. bizzozeronii* is lacking till now [8]. Animal and public health implications of *Helicobacter* species have been continuously debated since their introduction to the seroepidemiologic studies of *H. pylori* with transmission occurring from cats to humans [9] or from sheep milk to humans [10].

Recently, circumstantial evidence has suggested that these bacteria, also referred to as "*Helicobacter heilmannii*-like organisms" (HHLO), may be transmitted through animals [11]. At least two genotypically different types of HHLO have been identified based on the analysis of 16S rRNA genes, and type II was found to be highly related to *H. felis* and *H. bizzozeronii*. Although HHLO-2 (HHLO type II) was found in a small percentage of 0.2 to 0.6% in humans, approximately 50% of non-pylori *Helicobacter* species inducing gastritis, gastric ulcer and MALT lymphoma in humans are associated with HHLO-2 [12]. The infection with HHLO-2 is common in domestic animals with or without gastric diseases, and various studies have reported the prevalence rate as 41 to 100% in dogs and 57 to 100% in cats [13, 14]. The identification of HHLO-2 in humans that have mostly been identified in pets arouses interest in the zoonotic potential of HHLO-2; however, this finding is not clear as yet.

The aims of this study were to evaluate the prevalence and morphologic types of *Helicobacter* spp. using *Helicobacter* genus-specific PCR and *Helicobacter* species-specific PCR and to assess the relation of exposure to pets with HHLO-2 prevalence in a population-based study of pet owners in Korea.

## MATERIALS AND METHODS

*Study population and sample preparation*: One hundred and 24 human subjects were included in the "dog-contact risk factor group". In detail, the dog-contact risk factor group was composed of 41 dog owners and 43 veterinarians who regularly contact dogs. In this group, 12 veterinarians who were themselves dog owners. Each of the dog owners including veterinarians was matched with their own 39 dogs by labeling for the statistical analyses. Forty human subjects with no history of pet ownership were included for the negative dog-contact factor (Supplemental Fig. 1). Saliva and feces samples were taken from all subject populations. The feces and saliva samples were taken by using sterilized cotton swabs and subsequently submerged in 500 $\mu l$ of autoclaved phosphate buffered saline. DNA was extracted from 20 to 30 $\mu l$ of each sample by using DNeasy Tissue Kit (Qiagen, Santa Clarita, CA, U.S.A.). The DNA samples were eluted in 200 $\mu l$ volume and stored in a −20°C freezer until PCR was conducted.

*Genus-specific PCR*: Each DNA sample was amplified on *Helicobacter* 16S rRNA gene using C70 and B37 outer primers30, and subsequently, the PCR products were conducted nested PCR using C97 and C98 inner primer pair (Supplemental Table 1) [15]. The PCR mixture, total volume of 20 $\mu l$ contained a final primer concentration of 0.5 $\mu$M, 1 $\mu l$ DNA samples (0.3 $\mu l$ for nested PCR) and 18 $\mu l$ diethyl pyrocarbonate treated water, was added to Maxime PCR PreMix Kit (iNtRON Biotechnology Inc., Seoul, Korea). The PCR samples were heated at 95°C for 5 min followed by 30 cycles of denaturation at 94°C for 45 sec, annealing at 53°C for 45 sec and extension at 72°C for 3 min and finally extended at 72°C for 15 min using outer primers by using programmed temperature control system (PC808, Astec, Fukuoka, Japan). For nested PCR, the PCR products were heated at 94°C for 2.5 min followed by 35 cycles of denaturation at 94°C, annealing at 50.5°C, extension at 72°C for 1 min each and a final extension at 72°C for 15 min. The PCR products were electrophoresed by 1.5% agarose gels containing ethidium bromide in 0.5x TBE buffer and visualized on ultraviolet light illuminator.

*Species- specific PCR*: PCR amplifications of *H. felis* and *H. bizzozeronii* were performed using primer (Supplemental Table 1) which amplify the urease B gene of them [16]. PCR assay specific for *H. pylori* is also performed. The total volume of PCR mixture 20 $\mu l$ including a final each primer concentration of 0.5 $\mu$M, 1 $\mu l$ DNA samples (0.3 $\mu l$ for nested PCR) and 18 $\mu l$ diethyl pyrocarbonate treated water was added to Maxime PCR PreMix Kit (iNtRON Biotechnology Inc.). DNA extracts from pure cultures of *H. pylori*(HpKTCC H. pylori strain 114), *H. felis*(ATCC 49179) and *H. bizzozeronii*(ATCC 70030) served as positive controls.

For PCR amplification of *H. pylori*, the samples were heated at 95°C for 5 min and followed by 35 cycles at 94°C for 45 sec, at 59°C for 45 sec and at 72°C for 45 sec and a final extension at 72°C for 10 min using outer primes. Second round of PCR was performed at 95°C for 5 min, 30 cycles followed at 94°C for 45 sec, at 54°C for 45 sec and at 72°C for 30 sec and final extension at 72°C for 10 min. For *H. felis*-specific PCR, samples were heated at 94°C for 2.5 min once, followed by 40 cycles of denaturation at 94°C, annealing at 45°C, extension at 72°C for 1 min each with a final extension at 72°C for 15 min using outer primes. Second round of PCR was performed at 94°C for 2.5 min, 30 cycles followed at 94°C for 45 sec, at 50°C for 45 sec and at 72°C for 45 sec and final extension at 72°C for 15 min. The *H. bizzozeronii*-specific PCR was carried out following conditions, heated at 94°C for 2.5 min once and 33 cycles of at 94°C for 1 min, at 57°C for 1 min and at 72°C for 1 min. Final extension was performed at 72°C for 16 min using outer primes. Second round of PCR was performed at 94°C for 2.5 min, 30 cycles followed at 94°C for 45 sec, at 55.5°C for 45 sec and at 72°C for 45 sec and final extension at 72°C for 15 min. The PCR products were electrophoresed on ethidium-bromide stained 1.5% w/v agarose gels in 0.5x TBE buffer and visualized on ultraviolet light illuminator.

*Nucleotide sequence analysis*: In order to confirm the identity of *H. pylori*, *H. felis* and *H. bizzozeronii* specific PCR assay products with their target genes, after the PCR products of the specific size were extracted by commercial gel extraction kit (MEGAquick-spin, INTRON, Seoul, Korea), direct sequencing of the PCR products with specific primer was conducted by ABI Prism 3730 XL DNA Analyzer (PE Applied Biosystems, Foster City, CA, U.S.A.). The result of sequencing was compared to those present in databases using BLAST software.

Table 1. Transmitted infection possibility of *Helicobacter* spp., *Helicobacter pylori*, *Helicobacter felis* and *Helicobacter bizzozeronii* in dog-contact risk factor

| | | *Helicobacter* spp. [a] | | *Helicobacter pylori* [b] | | *Helicobacter felis* [c] | | *Helicobacter bizzozeronii* [d] | | Species Subtotal | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Negative | Positive | Negative | Positive | Negative | Positive | Negative | Positive | | |
| Dog-Contact | Negative | 19 | 21 | 34 | 6 | 39 | 1 | 24 | 16 | 40 | 160 |
| | Positive | 18 | 66 | 31 | 53 | 66 | 18 | 51 | 33 | 84 | 336 |

a) Pearson's $\chi^2$ test with Yates' continuity correction: $\chi^2$=7.59, $P$ <0.01, Fisher's exact test for count data: odds ratio=3.28, $P$ <0.01. b) Pearson's $\chi^2$ test with Yates' continuity correction: $\chi^2$=23.23, $P$=not significant, Fisher's exact test for count data: odds ratio=9.50, $P$=not significant. c) Pearson's $\chi^2$ test with Yates' continuity correction: $\chi^2$=6.09, $P$ <0.05, Fisher's exact test for count data: odds ratio=10.50, $P$ <0.01. d) Pearson's $\chi^2$ test with Yates' continuity correction: $\chi^2$=0, $P$=not significant, Fisher's exact test for count data: odds ratio=0.97, $P$=not significant.

Table 2. Transmitted infection possibility of *Helicobacter* spp., *Helicobacter pylori*, *Helicobacter felis* and *Helicobacter bizzozeronii* in veterinarian risk factor

| | | *Helicobacter* spp. [a] | | *Helicobacter pylori* [b] | | *Helicobacter felis* [c] | | *Helicobacter bizzozeronii* [d] | | Species Subtotal | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Negative | Positive | Negative | Positive | Negative | Positive | Negative | Positive | | |
| Veterinarian | Negative | 10 | 31 | 20 | 21 | 31 | 10 | 28 | 13 | 41 | 164 |
| | Positive | 8 | 35 | 11 | 32 | 35 | 8 | 23 | 20 | 43 | 172 |

a) Pearson's $\chi^2$ test with Yates' continuity correction: $\chi^2$=0.14, $P$=not significant, Fisher's exact test for count data: odds ratio=1.40, $P$=not significant. b) Pearson's $\chi^2$ test with Yates' continuity correction: $\chi^2$=3.59, $P$<0.05, Fisher's exact test for count data: odds ratio=2.73, $P$<0.05. c) Pearson's $\chi^2$ test with Yates' continuity correction: $\chi^2$=0.14, $P$=not significant, Fisher's exact test for count data: odds ratio=0.71, $P$=not significant. d) Pearson's $\chi^2$ test with Yates' continuity correction: $\chi^2$=1.35, $P$=not significant, Fisher's exact test for count data: odds ratio=1.85, $P$=not significant.

*Statistical analysis*: Pearson's chi-square test and Fisher's exact test were used to find the dependence between 2 categories. To check for the independence of 2 categories, including positive/negative PCR detection, the results in human subjects/animal subjects were tested by chi-square analysis using contingency tables. After counting the positive results for *Helicobacter* spp., *H. pylori, H. felis* and *H. bizzozeronii* as the variables, the possibility of risk of transmission between animal and human subjects was assessed using Pearson's chi-square test with Yates' continuity correction or 2-tailed Fisher's exact test for count data to rule out a variety of distribution assumptions. For this reason, Pearson's chi-square test is vague when cell counts are less than 5. From the contingency tables, odd ratios for each *Helicobacter* spp. can be calculated for interpreting the possible risk factor analysis. Statistical analyses were conducted using the conventional statistical software R (version 2.15.2), and the significance was set at $P$<0.05.

## RESULTS

*Risk factor analyses by Genus/species-specific PCR*: Genus/species-specific single PCR was performed using inner primer for 16S rRNA gene in *Helicobacter* spp. *H. pylori, H. felis* and *H. bizzozeronii* were detected by nested PCR assay (Supplemental Fig. 2). Direct sequencing was performed on 2 of each Helicobacter species-specific PCR product in both human and animal, and these PCR products were randomly selected. When the result of sequencing was compared to those present in databases using BLAST software, above 99% was represented homology to their specific *Helicobacter* species.

For the risk factor analyses, 124 eligible human subjects and 39 dogs participated in this study. Among the human subjects, 41 dog owners and 43 veterinarians including 12 veterinarians who were themselves dog owners were included in the statistical analysis. Relativity of contact with dogs and *Helicobacter* spp. infection diagnosed by genus-specific PCR showed a statistically significant result ($P$<0.01, Table 1), but in the relativity analyses between groups of humans with frequent contact with dogs and *H. pylori, H. felis* and *H. bizzozeronii* infections diagnosed using species-specific PCR, only *H. felis* showed a statistically significant result (Table 1). Although *H. pylori* infection showed a statistically significant relativity, no statistically significant association was found between the veterinarian subjects and *Helicobacter.* spp., *H. felis* and *H. bizzozeronii* infections (Table 2). On performing risk factor analyses of HHLO-2 infection by transmission, using matching species, between HHLO-2-positive dog owners and HHLO-2-positive dogs, *H. felis* infection showed an extremely significant relativity ($P$<0.0001, Table 3), and *H. bizzozeronii* may also be a possible significant risk factor ($P$<0.01, Table 3).

## DISCUSSION

The present study investigated the possible role of frequent contact with dogs or dog ownership in the transmission of *Helicobacter* spp. infection in a representative population sample comprising of dogs, dog owners, non-dog owners and veterinarians in Korea. We specifically tested the hypothesis that HHLO-2 may be a very important agent causing cross infection, because it has attracted attention as a zoonotic agent in recent studies [11, 17]. Dog owners and veterinarians were included as the subjects, because of their frequent contact with dogs. Overall, we found strong

Table 3. Risk factor analyses of *Helicobacter felis* and *Helicobacter bizzozeronii* by matching species between dog-owner and dog

| *Helicobacter felis* [a] | | Dogs | | *Helicobacter bizzozeronii* [b] | | Dogs | |
|---|---|---|---|---|---|---|---|
| | | Negative | Positive | | | Negative | Positive |
| Dog-owners | Negative | 23 | 0 | Dog-owners | Negative | 18 | 1 |
| | Positive | 7 | 9 | | Positive | 7 | 13 |

a) Pearson's $\chi^2$ test with Yates' continuity correction: $\chi^2$=13.79, $P$<0.0001, Fisher's exact test for count data: odds ratio=Inf*. *Infinite value describes the faulty cell data by excel program (0/0). b) Pearson's $\chi^2$ test with Yates' continuity correction: $\chi^2$=12.62, $P$<0.001, Fisher's exact test for count data: odds ratio=30.04, P <0.001.

evidence for an increased risk of HHLO-2 infections, such as *H. felis* and *H. bizzozeronii* infections, associated with the presence of dogs in the household (dog owners group), and co-infection rates were relatively high in dog-contact group (42.85%) than no dog-contact group (7.50%). In addition, *H. felis* and *H. bizzozeronii* co-infection rate was most frequent in both human and animal subject (42.85% in human subject and 55.12% in animal subject). In the previous study about *H. pylori*, co-infection rate of *H. pylori* L-form and vegetable-form in human subject was reported as 78.38% [19], but other form of *Helicobacter* spp. co-infection rate report is extremely rare. Results indicated that in the group that was in frequent contact with dogs inside or outside the house (group in frequent contact with dogs), genus *Helicobacter* spp. ($P$<0.01) and *H. felis*($P$<0.05) infection could be considered as a zoonotic infection. However, frequent contact with dogs did not have relativity as a risk factor in *H. pylori* and *H. bizzozeronii* infections, and this result supported the result of the previous studies [8]. Interestingly, the veterinary group in Korea showed a high prevalence of *H. pylori* infection with statistical significance as shown in Table 2.

Acquisition of HHLO-2, including *H. felis* or *H. bizzozeronii,* may occur through animal contact, contamination of the household environment and also provably through direct or indirect contact inside the house. Among these possible transmission routes, possibility of transmission via animal contact through the oral-oral or the fecal-oral route may be highest in dog owners as compared with non-dog owners. However, the other possible transmission routes are not apparent. A statistically strong positive relation between *H. felis* infection and contact with dogs was identified in this study. Therefore, the potential for zoonosis might be considered in *H. felis* infection, both from dogs to humans and from humans to dogs, indicating that dogs might be a source for this infection; and this result is in contrast with the result of previous studies that have considered a low possibility of zoonosis in *H. pylori* infection in Germany [8]. Further research should integrate the approaches to the environmental or cultural factors, including diet and other life-style variables with clear roles of individual factors.

Several reports described the isolation of *H. pylori* from cats, and a possible non-primate reservoir for *H. pylori* was also identified [13, 17, 18]. *H. pylori* has been discovered in the stomach of dogs, and *H. pylori* was found in 3 dogs with no clinical signs according to our PCR results (data not shown). This result may suggest that dogs could be the natural host for *H. pylori,* or it may be transmitted from other sources especially humans. Furthermore, on comparing the type of identified HHLO-2 in owners and their dogs, the dog owners group showed the presence of organisms identical to those in their dogs. It might be speculated that *H. felis* and *H. bizzozeronii* could be transmitted both from dogs to humans and from humans to dogs in a strong statistically significant manner. Although the other sources of infection, such as contaminated water or food, were considered, the possibility of these sources of infection is comparatively low in *H. pylori* infection. These data support that dog ownership was linked to a high socioeconomic status in the risk of transmitted HHLO-2 infection.

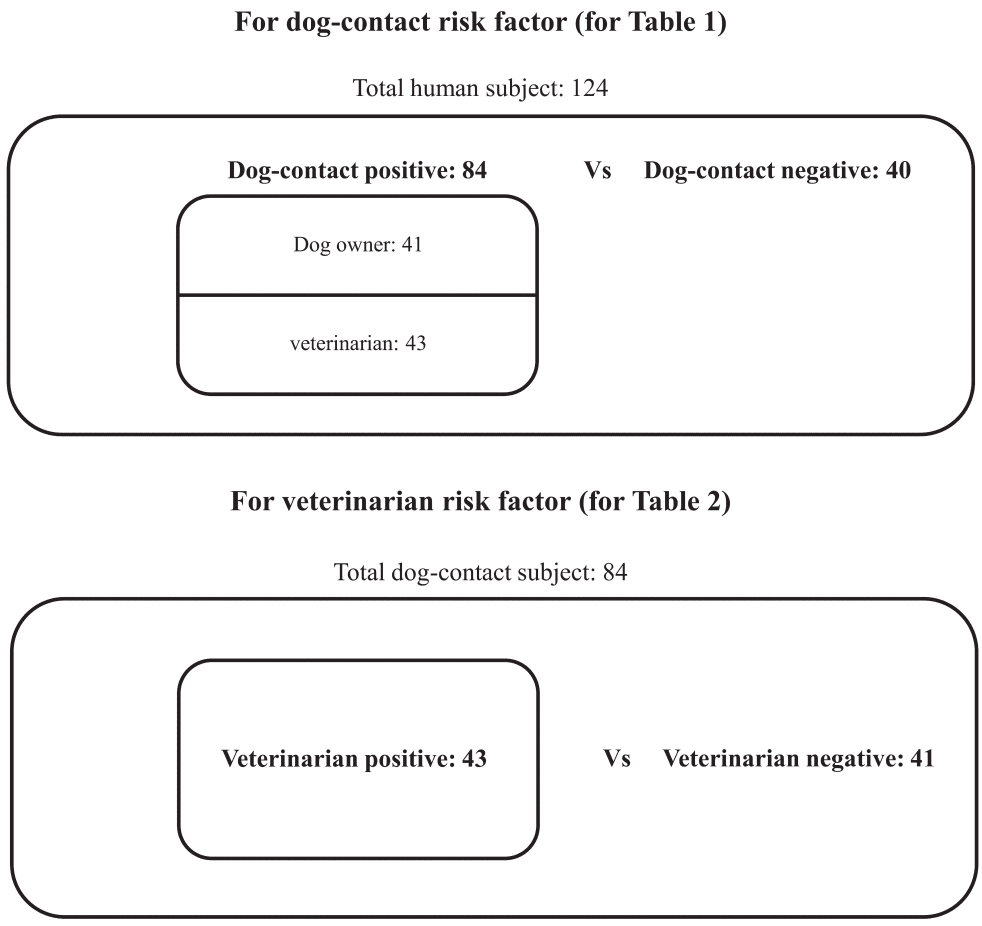
Treatment of *Helicobacter* infection consists of combination therapy with antimicrobial and anti-secretory drugs. It is already well-known that *Helicobacter* infection in dogs and cats can be eradicated with treatment, and it is also known which drugs are the best for use in the veterinary medical field. The exact details of transmission are still unclear. A strong incidence of HHLO-2 infection has been reported in dog owners, suggesting that transmission from animals to humans or from humans to animals is strongly possible. In this report, we have statistically significant supporting results in dog owners and veterinarians of HHLO-2 infection, which should be included as the subjects, because of their frequent contact with dogs. Overall, we found strong evidence for an increased risk of HHLO-2 infections, such as *H. felis* and *H. bizzozeronii* infections from this research, although infection-positive subjects showed no clinical signs (subclinical infection) mostly. Only few human subjects reported extremely sporadic vomit symptoms in the pre-investigation for sampling procedures.

In conclusion, we might suggest that HHLO-2 (*Helicobacter heilmannii*-like organisms type 2) infection might be zoonotic, because continuous contact with dogs was proved to be correlated with human *H. felis* and *H. bizzozeronii* infections in this study. However, this report never intended to criticize pet ownership, but to carry out more intensive prevention, treatment and socio-epidemologic research of *Helicobacter felis* and *H. bizzozeronii* infections, which should be considered in both the medical and veterinary fields.

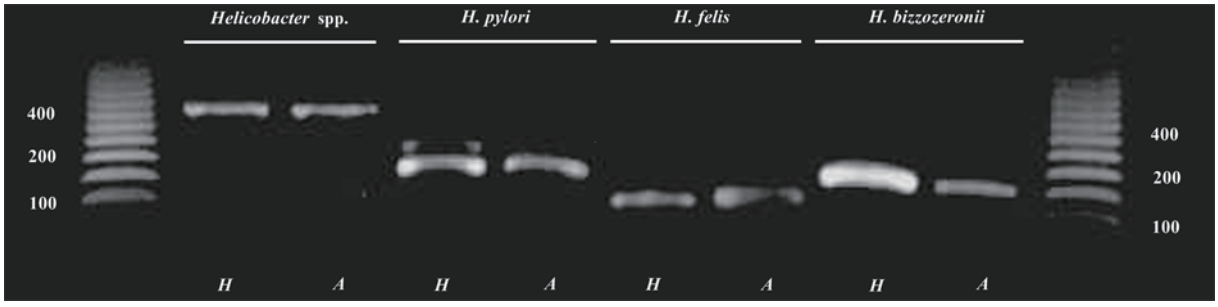
## REFERENCES

1. Ansorg, R. and Von Recklinghausen, G. 1995. Cat Owners' Risk of Acquiring a *Helicobacter pylori* infection. *Zentralbl. Bakteriol. Mikrobiol.* **283**: 122–126. [CrossRef]
2. Bode, G., Rothenbacher, D., Brenner, H. and Adler, G. 1998.

Pets are not a risk factor for *Helicobacter pylori* infection in young children: results of a population-based study in Southern Germany. *Pediatr. Infect. Dis. J.* **17**: 909–912. [Medline] [CrossRef]
3. De Bock, M., Decostere, A., Hellemans, A., Haesebrouck, F. and Ducatelle, R. 2006. *Helicobacter felis* and *Helicobacter bizzozeronii* induce gastric parietal cell loss in Mongolian gerbils. *Microbes. Infect.* **8**: 503–510. [Medline] [CrossRef]
4. Dore, M. P., Sepulveda, A. R., El-Zimaity, H., Yamaoka, Y., Osato, M. S., Mototsugu, K., Nieddu, A. M., Realdi, G. and Graham, D. Y. 2001. Isolation of *Helicobacter pylori* from sheep—implications for transmission to humans. *Am. J. Gastroenterol.* **96**: 1396–1401. [Medline]
5. Fox, J. G., Batchelder, M., Marini, R., Yan, L., Handt, L., Li, X., Shames, B., Hayward, A., Campbell, J. and Murphy, J. 1995. *Helicobacter pylori*-induced gastritis in the domestic cat. *Infect. Immun.* **63**: 2674–2681. [Medline]
6. Fox, J. G., Dewhirst, F. E., Shen, Z., Feng, Y., Taylor, N. S., Paster, B. J., Ericson, R. L., Lau, C. N., Correa, P. and Araya, J. C. 1998. Hepatic *Helicobacter* species identified in bile and gallbladder tissue from chileans with chronic cholecystitis. *Gastroenterology* **114**: 755–763. [Medline] [CrossRef]
7. Ghil, H. M., Yoo, J. H., Jung, W. S., Chung, T. H., Youn, H. Y. and Hwang, C. Y. 2009. Survey of *Helicobacter* infection in domestic and feral cats in Korea. *J. Vet. Sci.* **10**: 67–72. [Medline] [CrossRef]
8. Ha, S. W., Chung, T. H., Youn, H. Y. and Hwang, C. Y. 2008. PCR based detection of *Helicobacter* spp. in Saliva, Dental plaque, vomitus and feces of dogs. *J. Vet. Clin.* **25**: 447–451.
9. Handt, L. K., Fox, J. G., Dewhirst, F. E., Fraser, G. J., Paster, B. J., Yan, L. L., Rozmiarek, H., Rufo, R. and Stalis, I. H. 1994. *Helicobacter pylori* isolated from the domestic cat: public health implications. *Infect. Immun.* **62**: 2367–2374. [Medline]
10. Nakamura, M., Matsui, H., Akiba, Y., Nishikawa, K., Takahashi, S. and Ishii, H. 2003. Relationship of *Helicobacter heilmannii* Infection and Parietal Cell. *Prog. Med.* **23**: 822–825.
11. Prachasilpchai, W., Nuanualsuwan, S., Chatsuwan, T., Techangamsuwan, S., Wangnaitham, S. and Sailasuta, A. 2007. Diagnosis of *Helicobacter* spp. infection in canine stomach. *J. Vet. Sci*. **8**: 139–145. [Medline] [CrossRef]
12. Priestnall, S. L., Wiinberg, B., Spohr, A., Neuhaus, B., Kuffer, M., Wiedmann, M. and Simpson, K. W. 2004. Evaluation of "*Helicobacter heilmannii*" subtypes in the gastric mucosas of cats and dogs. *J. Clin. Microbiol.* **42**: 2144–2151. [Medline] [CrossRef]
13. Ricci, C., Holton, J. and Vaira, D. 2007. Diagnosis of *Helicobacter pylori*: Invasive and non-invasive tests. *Best. Pract. Res. Clin. Gastroenterol.* **21**: 299–313. [Medline] [CrossRef]
14. Simpson, K. W., Strauss-Ayali, D., McDonough, P. L., Chang, Y. F. and Valentine, B. A. 1999. Gastric function in dogs with naturally acquired gastric *Helicobacter* spp. infection. *J. Vet. Intern. Med.* **13**: 507–515. [Medline]
15. Song, Q., Lange, T., Spahr, A., Adler, G. and Bode, G. 2000. Characteristic distribution pattern of *Helicobacter pylori* in dental plaque and saliva detected with nested PCR. *J. Med. Microbiol.* **49**: 349–353. [Medline]
16. Stolte, M., Wellens, E., Bethke, B., Ritter, M. and Eidt, H. 1994. *Helicobacter heilmannii* (formerly *Gastrospirillum hominis*) gastritis: an infection transmitted by animals? *Scand. J. Gastroenterol.* **29**: 1061–1064. [Medline] [CrossRef]
17. Trebesius, K., Adler, K., Vieth, M., Stolte, M. and Haas, R. 2001. Specific detection and prevalence of *Helicobacter heilmannii*-like organisms in the human gastric mucosa by fluorescent *in situ* hybridization and partial 16S ribosomal DNA sequencing. *J. Clin. Microbiol.* **39**: 1510–1516. [Medline] [CrossRef]
18. Vinette, K. M., Gibney, K. M., Proujansky, R. and Fawcett, P. T. 2004. Comparison of PCR and clinical laboratory tests for diagnosing *H. pylori* infection in pediatric patients. *BMC Microbiol.* **4**: 5. [Medline] [CrossRef]
19. Wang, K. X., Li, C. P., Cui, Y. B., Tian, Y. and Yang, Q. G. 2003. L-forms of *H. pylori*. *World J. Gastroenterol* **9**: 525–528. [Medline]




Supplemental Fig 1. Study population.




Supplemental Fig. 2. Detection of *Helicobacter* spp. DNA by genus-specific and species-specific nested PCR with specific primer (representative): Lanes 1 and 2 *Helicobacter* spp. genus positive samples (*H.* spp.) in human subject (*H*) and animal subject (*A*) (400 bp), lanes 3 and 4 *H. pylori* positive samples in human subject (*H*) and animal subject (*A*) (230 bp), lanes 5 and 6 *H. felis* positive samples in human subject (*H*) and animal subject (*A*) (160 bp), lanes 7 and 8 *H. bizzozeronii* positive samples in human subject (*H*) and animal subject (*A*) (207 bp) and each side marker (M); 50 bp DNA ladder.

Supplemental Table 1. Primer sequences used for Helicobacter spp. polymerase chain reaction

| Target Gene | Species | Oligonucleotides of PCR primer | Products (bp) | Reference Article No. |
|---|---|---|---|---|
| 16S rRNA | *Helicobacter* spp. | Outer primer<br>*F*: 5′-AGA GTT TGA TYM TGG C-3′<br>*R*: 5′-TAC GGY TAC CTT GTT ACG A-3′ | 1,506 | [15] |
| | | Inner primer<br>*F*: 5′-GCT ATG ACG GGT ATC C-3′<br>*R*: 5′-GAT TTT ACC CCT ACA CCA-3′ | 400 | |
| Urease B | *H. pylori* | Outer primer<br>*F*: 5′-CCC TCA CGC CAT CAG TCC CAA AAA-3′<br>*R*: 5′-AAG AAG TCA AAA ACG CCC CAA AAC-3′ | 417 | [15] |
| | | Inner primer<br>*F*: 5′-GGC AAA TCA TAA GTC CGC AGA A-3′<br>*R*: 5′-TGA GAC TTT CCT AGA AGC GGT GTT-3′ | 230 | |
| | *H. felis* | Outer primer<br>*F*: 5′-ATG AAA CTA ACG CCT AAA GAA CTA G-3′<br>*R*: 5′-GGA GAG ATA AAG TGA ATA TGC GT-3′ | 1,150 | [12] |
| | | Inner primer<br>*F*: 5′-TGT TAG ACT CGG CGA CAC TG-3′<br>*R*: 5′-GGC GTT AGT GAG CAC ACC AT-3′ | 160 | |
| | *H. bizzozeronii* | Outer primer<br>*F*: 5′-GAA GTC GAA CAT GAC TGC AC-3′<br>*R*: 5′-GGT CGC ATT AGT CCC ATC AG-3′ | 420 | [12] |
| | | Inner primer<br>*F*: 5′-GGG ATG GCA CAA ACC AAT AG-3′<br>*R*: 5′-AGC CAA AGC CTC AGT AGC AG-3′ | 207 | |

*F: forward, R: reverse*