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Original Article

Seroprevalence of *Helicobacter pylori* associated with gastrointestinal implication in pet dogs of Urmia, Iran

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Summary
Helicobacter pylori (H. pylori) is a Gram-negative microaerophilic, curved to spiral-shaped, motile bacterium capable of infecting humans and several animal species. The aim of this study was to quantify immunoglobulin G (IgG) antibodies to *H. pylori* among pet dogs in relation to gastrointestinal disturbances in Urmia (west Azerbaijan, Iran). Blood samples of 87 pet dogs (64 males and 23 females) were collected via their cephalic vein. Antibodies (IgG) against canine *H. pylori* were detected by a commercial enzyme-linked immunoassay assay (ELISA) utilizing a monoclonal anti-Hp-IgG antibody and Hp-IgGHRP conjugate. The results showed that 59 male dogs out of 64 and all 23 female dogs had an IgG levels greater than 100 ng/mL (94.2%). The mean (± SD) was 131 ± 10.69 ng/mL. The rest of the dogs (5 male dogs) showed IgG values below 60 ng/mL (6.8%). The mean (± SD) was 52 ± 8.52 ng/mL. Sifting through the medical backgrounds and data of the dogs showed that 63 out of 82 dogs (76.8%) with high IgG values, had a current or past history of gastrointestinal problems. The statistical analysis showed no significant difference in the prevalence of seropositive cases within different sexes and age groups ($p > 0.05$). The current study is the first to address the quantification of IgG titers for *H. pylori* in dogs in the region, showing its possible strong connection with gastrointestinal disturbances, which could thus be considered a step forward in deciphering rather complex epidemiology of *H. pylori.*
Keywords: dogs, *Helicobacter pylori*, ELISA, IgG

Introduction

Helicobacter pylori (*H. pylori)* is a Gram-negative microaerophilic, curved to a spiral-shaped, motile bacterium, which was first described by Warren and Marshall in 1983 in association with chronic gastritis and peptic ulcer. Later, they received Nobel Prize for their achievement (Goodman et al., 2008). After almost three decades, it has now been confirmed that *H. pylori* is capable of causing a wide variety of gastrointestinal disorders in humans, including chronic gastritis, gastro-duodenal ulcerations, gastric MALT (mucosa-associated lymphoid tissue) lymphoma or cancer (Wang et al., 2014; Salar, 2019).

Growing evidence reported that isolation of *H. pylori* from domestic animals, such as cats, dogs, and sheep points that *H. pylori* infection may probably be transmitted from these domestic

animals to humans and vice-versa. In this regard, a previous study in the Columbian Andes presented an excessive *H. pylori* infection in children having contact with sheep (Goodman et al., 1997). Moreover, another similar study on Sardinian shepherds with close contact with sheep and school children from rural areas of Sardinia, proposed that contact with these domestic animals may be a remarkable and independent risk factor for *H. pylori* infection in this Sardinian subpopulation (Papiez et al., 2003). On the other hand, *H. pylori* colonizes half of the world's population, whose 70% are asymptomatic. This high rate of colonization is in stark contrast with the apparently low colonization rate in animals, posing a likely scenario of "reverse zoonosis" (Vale and Vítor, 2010).

There are also some data indicating helicobacter pathogenicity in domestic animals. Esteves et al. (2000) showed that infected cats with *H. pylori* developed a lymphofollicular gastritis with small to moderate numbers of eosinophils and a moderate antral infiltration of neutrophils. In another study, 88% of the infected cats exhibited moderate to severe lymphofollicular gastritis (Handt et al., 1995). Of note, gastritis with the lymphofollicular feature in both cats and people is typical of Helicobacter-induced gastritis. The affected cats may show the clinical symptoms, such as chronic vomiting, weight loss, and possibly diarrhea, though infections are frequently subclinical. Dogs can be experimentally infected, but there is limited document of gastrointestinal pathology in dogs. Besides, it seems that many, if not most, *Helicobacter spp*. recovered from naturally infected dogs are species other than *H. pylori*, e.g., *H. bizzozeronii* (Neiger and Simpson, 2000).

The aim of this study was to quantify IgG antibodies to *H. pylori* among pet dogs in relation to gastrointestinal disturbances in Urmia (west Azerbaijan, Iran). The degree of their antibodies' titer can not only provide a better picture of this bacterium status in this species, but also could be interpreted as regards the potential infection of human households, who are in close contact with their animals.

Materials and Methods

Between August 2018 and March 2019, blood samples of 87 pet dogs (64 males and 23 females), which were attended the Veterinary Teaching Hospital (Faculty of Veterinary Medicine-Urmia University, Iran) for various reasons, were collected via their cephalic vein. The blood sera were separated and stored in a freezer at -70°C until further analysis.

Antibodies (IgG) against canine *H. pylori* were detected by a commercial enzyme-linked immunosorbent assay (ELISA) technique, utilizing an HRP (horseradish peroxidase)-conjugated monoclonal anti-IgG (BlueGene Biotech, China).

In brief, the assay proceeded according to the following: 1) the serum samples were diluted 1 to 200 (5 µL + 995 µL) with the diluent buffer; 2) a 100 µL of the blank solution (diluent buffer), the calibrator, the control, and the diluted sample were pipetted into the wells of a microplate adsorbed with purified bacterial antigen; 3) after incubation for 30 min at 37°C, the wells were washed three times with the washing buffer and 100 µL of the conjugate solution (HRP-conjugated monoclonal anti-IgG) was added into the wells; 4) they were incubated for 30 min at 37°C and again were washed for three times; 5) a 100 µL of the mixed substrate solution containing tetramethylbenzidine was added into the wells and they were incubated for further 30 min at room temperature (20°C - 25°C); 6) The reaction was stopped by adding 100 µL of the stop solution into the wells; 7) the intensity of color was measured spectrophotometrically at 450 nm in a microplate reader; 8) a standard curve was constructed by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis. The concentrations of the samples corresponding to the mean absorbance were calculated from the standard curve, 8) The cut-off value was calculated by multiplying the calibrator O.D. by calibrator factor (CF), 9) Immune-Status-Ratio (ISR) or antibody index was calculated by dividing the O.D. value of each sample by cut-off value. If the index was ≤ 0.90

(corresponding 76 ng/mL in terms of the antibody concentration), it was considered negative. When the index was ≥1.10 (corresponding 90 ng/mL in terms of the antibody concentration), it was considered as positive.

The results were statistically analyzed using Fisher's exact test to compare the relative frequency of seropositive cases among different gender and age groups (i.e., less or greater than 2.5 years). The software of SPSS (version 20, Armonk, NY) was employed for the analysis. A *p* value of equal or less than 0.05 was considered statistically significant.

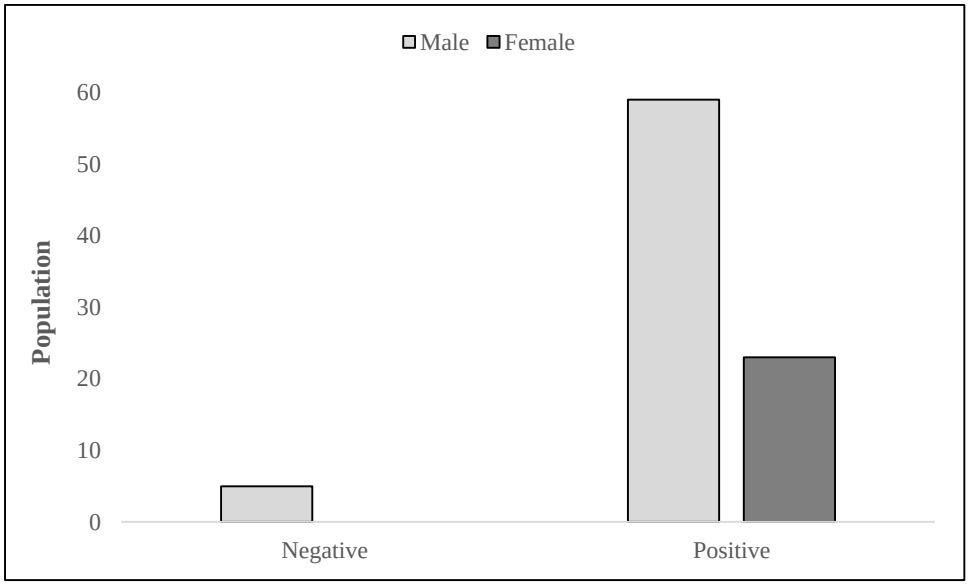

Fig. 1. Comparison of *H. pylori* prevalence in different sexes. No significant difference was observed in prevalence of seropositive cases between male and female dogs ($p > 0.05$).

Results

The results showed that 59 male dogs out of 64 and all 23 female dogs showed an IgG level greater than 100 ng/mL (94.2%; Figure 1). The mean (± SD) was 131 ± 10.69 ng/mL. The rest of the dogs (five male dogs) showed IgG values below 60 ng/mL (6.8%). The mean (± SD) was 52 ± 8.52 ng/mL. Four dogs with lower IgG were under 2.5 years old and one was older than that (Figure 2). No significant difference was observed in the prevalence of seropositive cases between male and female dogs ($p > 0.05$). Similarly, the age did not appear to have any significant effect on the seroprevalence of *H. pylori* ($p > 0.05$).

Sifting through the medical backgrounds and data of the dogs showed that 63 out of 82 dogs (76.8%) with high IgG values, had a current or past history of gastrointestinal problems. No gastrointestinal disturbances were found in the history of five males with lower IgG values.

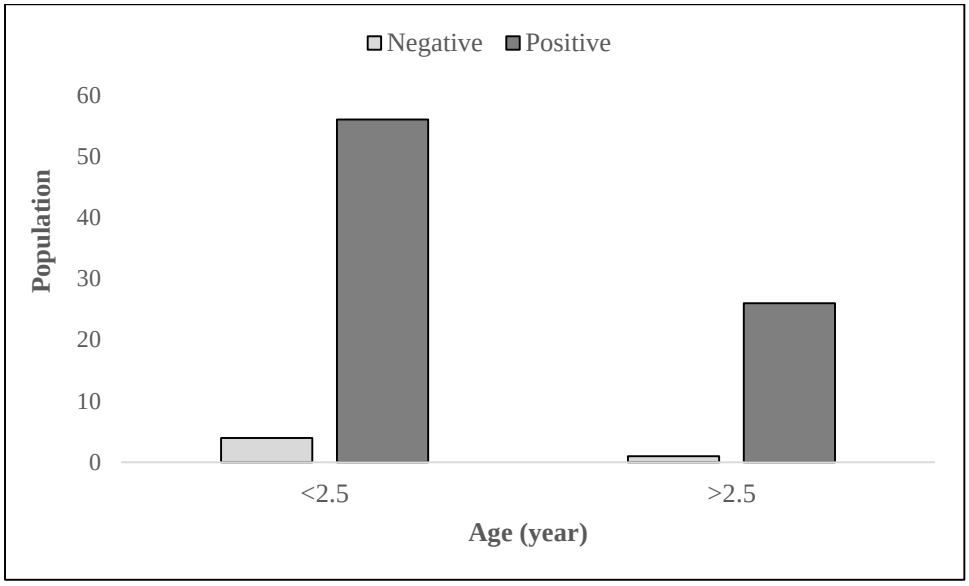

Fig. 2. Comparison of *H. pylori* prevalence in different age groups. The age did not have any significant effect on seroprevalance of *H. pylori* ($p > 0.05$).

Discussion

In this study, we quantified the seroprevalence of *H. pylori* in dogs. The occurrence of *Helicobacter spp.* in the stomachs of dogs and cats has long been studied earlier (Neiger and Simpson, 2000). It was reported that 67% to 86% of clinically healthy dogs and 61% to 100% of dogs presented chronic vomiting. By contrast, spiral-shaped organisms have been detected in cats in 41% to 100% of the animals detected, with a slightly higher rate in animals having chronic vomiting. However, the pathogenic significance of these *Helicobacter spp.* still remains enigmatic. Most species isolated by now from dogs and cats' stomach are *Helicobacter felis*, *Helicobacter heilmannii, Helicobacter bizzozeronii*, and to a lesser degree *H. suis*. However, incidental isolation of *H. pylori* from stomachs of dogs and particularly cats has also been reported (Neiger and Simpson, 2000). Although, *H. pylori* in humans has long been associated with various gastric disorders, its role in inducing abnormality in the stomachs of dogs and cats remains unclear and controversial. As mentioned before, multifocal gastritis and moderate to severe lymphofollicular gastritis in cats have been associated with *H. pylori*. Interestingly, feline isolates of *H. pylori* are genetically similar (99.7% sequence identity in 16S rRNA) to human isolates (Handt et al., 1995). Some reports have mentioned that animals are unlikely to play an important role in transmission of *H. pylori* to humans. However, a set of different research has demonstrated that close contact with animals can dramatically increase the prevalence rate (Moussa et al., 2021). In a Polish study conducted by Papiez and colleagues (2003), the *H. pylori* prevalence reached 97.6% in shepherds and 86% in their family members, but significantly less (65.1%), in the controls without contact with sheep. That study could bring some strong evidence that *H. pylori* could be considered a potent zoonosis, challenging previous suggestions of it as "reverse zoonosis". The presence of pets in the human household has also been the focus of attention in some studies. Surprisingly, the presence of pets in the household was demonstrated to decrease the risk of *H. pylori* seropositivity in both symptomatic and asymptomatic children. At present, there is not a good explanation for these observations (Chong et al., 2003). An earlier study in the US also reported similar findings that a lower *H. pylori*

seropositivity rate was associated with families owning dogs or cats (Brown, 2000). In another study, McIsaac and Leung (1999) investigated that no association was found between pet ownership and a history of peptic ulcer disease in 15,779 Canadian adults. On the other hand, the rest of *helicobacter spp.* found in animals can also be transmitted to humans. The data also show that *H. suis* is the most prevalent gastric non-*H. pylori* in humans and that there are strong indications that pigs may be a source of infection and likely a gastric pathogen in humans, too (Rimbara et al., 2021). Several reports indeed suggest the transmission of gastric non-*H. pylori* spiral bacteria from dogs and cats to humans (Loon et al., 2003; Baele et al., 2009). In spite of all these studies, a very recent paper by Kubota-Aizawa et al. (2021) has confirmed the co-infection of the owner and the two dogs with a genetically identical *H. pylori* strain, adding more weight to the importance of our findings. In our study, out of 89 dogs, 84 of either sex had higher IgG for *H. pylori*. Moreover, later scrutiny of the medical records of these dogs showed that 76.8% of them at some point experienced some gastrointestinal disturbances, which mostly manifested by gastritis and vomiting. No gastrointestinal disturbance was found in the history of the dogs with the lower titers. The current pattern shows a high degree of prevalence among mostly pet dogs for *H. pylori* without any discrimination towards age or sex. We did not study the status of *H. pylori* in the relevant household, which could be the focus of future research. We were also unable to track down the source of infection for the animals. In human studies, there are very strong relationships between the poorer socioeconomic status of households and the higher prevalence and incidence of *H. pylori* (Moayyedi et al., 2002). Nonetheless, most pet owners in Iran hail from well-to-do families and one could expect a higher degree of hygiene among their pets, too. There are several pieces of evidence that support a gastro–oral, oral–oral and fecal–oral transmission (Dowsett and Kowolik, 2003). Transmission may occur in a vertical mode (e.g., from parents to offspring) or in a horizontal mode (across individuals or from environmental contamination). In either case, the involvement of water and food cannot be excluded as vehicles or sources of infection (Zamani et al., 2017). However, it seems there is quite a different pattern for these between rural and urban areas. In developed areas, person-to-person transmission within families appears to be dominant, while in rural developing areas, the transmission pathway appears to be more complex (Vale and Vítor, 2010). Thus, it can be postulated that all seroconversion of our study might have been originated from human sources following the pattern of transmission in urban/developed areas. Nevertheless, the infection might have come from a common infected food source as several studies suggest milk or meat can infect people (Ghasemian Safaei et al., 2011; Zamani et al., 2017). One can also argue that this high prevalence of *H. pylori* might have been caused by high cross-reactivity among different *Helicobacter spp.,* which may naturally harbor in the stomach of the concerned animals. Although, we could not rule out this possibility, this could not diminish the importance of our study, as a range of *Helicobacter* species may also infect humans, resulting in gastritis, peptic and duodenal ulcers, and low-grade mucosa-associated lymphoid tissue lymphoma. However, this limitation should have also been overcome if the PCR test had been used to determine the type of bacteria species before the ELISA test. Further molecular and culture studies would help to identify and distinguish them at the species level. Plus, further elucidating these research questions will require large population cohort studies incorporating various complementary standardized detection methods.

Conclusion

In conclusion, the current study is the first to address the quantification of IgG titers for *H. pylori* in dogs, showing its possible strong connection with gastrointestinal disturbances, which could thus be considered a step forward in deciphering rather complex epidemiology of *H. pylori.* Additionally, the current work sheds more light on

the bacterium's distribution, which could be necessary for public health measures, especially among people associated with animals.

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Ethical Approval

This study was approved by the Research & Ethics Committee of the Faculty of Veterinary Medicine of Urmia University according to the current laws and regulations (No. 1224).

Conflict of interest statement

The authors declare no conflict of interest.

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