MAE Vet Fak Derg, 8 (2): 69-73, 2023
DOI: 10.24880/maeuvfd.1218001

Research Article

Determination of *Helicobacter heilmannii* in cats by real time polymerase chain reaction and histopathology

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Key Words:
cat
histopathology
real-time PCR
H. heilmannii

Received : 12.12.2022
Accepted : 31.07.2023
Published Online : 31.08.2023
Article Code : 1218001

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ABSTRACT

In recent years, many *Helicobacter* species have been identified in domestic animals and researches on identifying these species as zoonotic agents are increasing. The aim of this study is to reveal the presence of *H.heilmannii* in stomach and liver tissues taken during necropsies of domestic, stray and shelter cats by comparing the histopathological findings and Real-time PCR results. The material of the study consisted of stomach and liver tissues taken from 41 cats who died from different causes and were necropsied. DNA of *H. heilmannii* was determined in the stomach tissues of 36 (87.8%) cats and the liver tissues of 24 (58.5%) cats in the study conducted with Real-time PCR using specific primers of *H. heilmannii*. Histopathologically, degeneration, desquamation and necrosis in the gastric epithelium, fibrosis and edema in the lamina propria, and lymphoplasmatic cell infiltration were detected in cats diagnosed with gastritis. Eight cats were positive in Hematoxylin-Eosin staining and nineteen cats were positive in the staining with Warthin Starry of the sections, in terms of Helicobacter-like microorganisms. Microscopically, dissociation in the remark cords, hydropic degeneration in hepatocytes, and focal mononuclear cell infiltrations in some sections were determined in the livers. In conclusion, the detection of *H. heilmannii* at the rate of 87.8% in cat stomachs by Real-time PCR revealed a high prevalence of *H. heilmannii* in cats in the Konya region of Turkey. In addition, it was concluded that histopathological examinations are necessary to correlate the presence of bacteria with the disease state.

INTRODUCTION

Helicobacter heilmannii is a 4 to 10 µm long spiral-shaped, three to eight helical, flagellated and motile bacterium previously known as *"Gastrospirillium hominis"* (Murray et al., 1995). According to reports, studies on cats usually identify the *H. heilmannii, H. pylori, H. felis, H. bizzozeronii,* and *H. salomonis* species (Kubota-Aizawa et al., 2017; Bruyette, 2020; Matos et al., 2020). These agents have also been identified in human gastric biopsies, and studies have been published stating that they may be zoonotic (Haesebrouck et al., 2009; Tabrizi et al., 2015; Blois, 2020). It is known that *H. heilmannii* colonizes the stomach mucosa of cats, with detection rates varying from 57% to 100% (Hong et al., 2016).

A sequence similarity of 99% was determined between *Helicobacter* species isolated from cats suffering from gastric disease and those isolated from humans (Kubota-Aizawa et al., 2017). *Helicobacter* species were positive in 40% of nodular gastritis cases in humans, 24% of MALT lymphoma cases, 17% of chronic gastritis and 33% of gastroduodenal ulcer cases (Nakamura et al., 2020). *H. heilmannii* has been detected in stomach biopsies of patients with gastric symptoms at rates ranging from 8% to 19% (Bahadori et al., 2018; Matos et al., 2020).

Although *H. heilmannii* has been associated with chronic active gastritis, its pathogenicity in cats and dogs in terms of gastritis, peptic ulceration, and chronic vomiting remains unclear (Matos et al., 2020). Researchers reported that the majority of *Helicobacter* infections in cats and dogs were asymptomatic (Otto et al., 1994; Eaton et al., 1996; Norris et al., 1999). In cases of gastritis of different severity associated with *Helicobacter* species, lymphocyte and plasma cell infiltrations with eosinophil leukocytes have been reported. It has also been described that the stomach glands were enlarged, and fibrosis was determined microscopically (Erginsoy et al., 2006). Husnik et al. (2022) stated that although *Helicobacter* species were identified during microscopic examinations of the stomachs of dogs, the presence of *Helicobacter* species was not associated with the severity of the inflammation.

Histopathology, cytology, bacterial isolation and identification, polymerase chain reaction (PCR) techniques based on the replication of the genomic DNA of the agent, and serological techniques are the main methods used in studies aiming the detection of *Helicobacter* species in cats and dogs (Happonen et al., 1996; Neiger et al., 1999). The aim of this study is to reveal by Real-time PCR and histopathological methods the presence of *H. heilmannii* in stomach and liver tissues taken during necropsy from domestic, stray and shelter cats, which died from different causes, and to compare histopathological

To cite this article:
Tuzcu N, Celik Z, Akcakavak G, Bulut A, Salık R, Oner M, Kaya V, Tuzcu M. Determination of *Helicobacter heilmannii* in cats by real time polymerase chain reaction and histopathology. MAE Vet Fak Derg. 2023; 8 (2) :69-73.

findings with Real-time PCR results.

MATERIAL and METHODS

Animal materials

The stomach and liver tissues of 41 cats, who died in 2019-2022 for different reasons and were brought to the Department of Pathology, Faculty of Veterinary Medicine, Selcuk University for necropsy, constituted the material of the study. Eleven of the cats examined in the study were from the shelter, and twenty, stray cats brought in by municipal officials and animal lovers. Ten of the cats were domestic cats. Twenty-four of the studied cats were female, and seventeen were male. The related study was approved by SÜVDAMEK (Decision no: 2022/75).

Histopathological examination

Stomach and liver samples taken for histopathological examinations were fixed in a 10% buffered formalin solution, and routine tissue follow-up was performed. The obtained sections were stained with Hematoxylin-Eosin (HE) and Warthin Starry (WS) methods (Luna, 1968). The prepared sections were examined under a light microscope (Olympus BX51, Tokyo, Japan).

Real-time PCR (qPCR) examination

Tissue samples taken from the antrum region of the stomach and the right lobe of the liver were stored at -20°C prior to Real-time PCR examinations. The DNA isolation from stomach and liver samples was performed using a commercial DNA isolation kit (Roche, MagNA Pure LC DNA, Cat No; 03264785001). The obtained DNAs were stored at -20°C. The DNA copies of *H. heilmannii* were detected with a Real-time PCR device (Light Cycler 2.0, Roche) using primer probes prepared by a private company. Primer sequences used in Real-time PCR analysis were F: GGG CGA TAA AGT GCG CTT G, R: CTG GTC AAT GAG AGC AGG (Neiger ve ark 1998). Deionized water was used as the negative control.

RESULTS

Macroscopically, bronchopneumonia was found in 9 cats, diaphragmatic hernia in 2 cats, myocarditis in 3 cats, trauma symptoms in 15 cats, and gastroenteritis in 20 cats. Microscopic examination of samples taken from the antrum region of the stomachs revealed degeneration, desquamation and necrosis of the mucosa and glandular epithelium in ten cats. In addition, lamina propria edema was observed in these cats. Mononuclear cell infiltration and fibrosis in the lamina propria were detected in four stomach samples (Figure 1.A). Eight cats were found to be positive for Helicobacter-like microorganisms in the HE staining of the sections and nineteen cats in the WS staining (Figure 1.B). Microscopic examination of liver samples were determined dissociation of remark cords and hydropic degeneration of hepatocytes in nineteen cats. Focal mononuclear cell infiltrations were observed in portal area in four of the livers examined (Figure 1.C). Helicobacter-like microorganisms could not be detected in HE and WS staining of liver sections.

DNA of *H. heilmannii* was detected in stomach by Real-time PCR in 36 cats. In the study, *H. heilmannii* rates determined by Real-time PCR in domestic, stray and shelter cats 80 % (8/10) , 90% (18/20) and 90,9% (10/11), respectively. The cats identified with *H. heilmannii* by Real-time PCR, 44.44% (16/36) were male, and 55.55% (20/36) were female. According to age, 22.22% (8/36) were younger than one year old, 44.44% (16/36) were between 1-3 years old, and 33.33% (12/36) were older than 3 years.

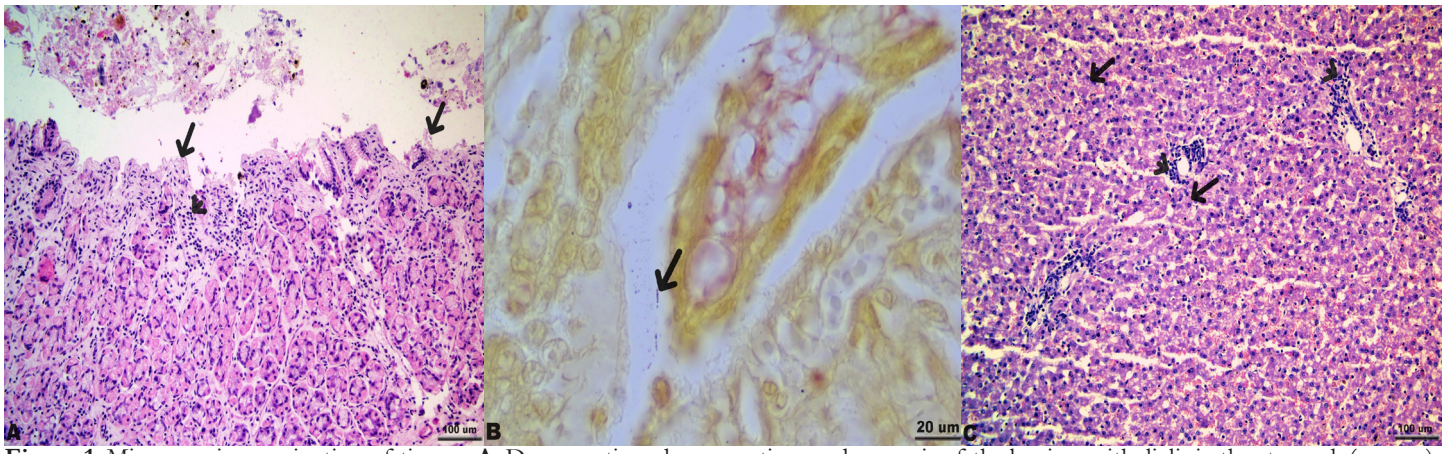

Figure 1. Microscopic examination of tissues. **A.** Degeneration, desquamation, and necrosis of the lamina epithelialis in the stomach (arrows), inflammatory cell infiltration (arrowhead), HE, x200. **B.** Helicobacter-like microorganism (arrow), WS, x1000. **C.** Hydropic degeneration (arrows) and focal inflammatory cell infiltration (arrowheads) of liver parenchyma, HE, x200.

The DNA of *H. heilmannii* was detected in liver samples with Real-time PCR in 24 cats using *H. heilmannii*-specific primers (Figure 2). Real-time PCR results showed that male cats constituted 45.83% (11/24) of cats with *H. heilmannii* found in liver samples, and female cats, 54.16% (13/24). Cats with copies of *H. heilmannii* DNA in their liver 25% (6/24) were less than one year old, 41.6% (10/24) were between 1-3 years old, and 33.3% (8/24) were older than 3 years.

DNA of *H. heilmannii* was determined in the stomach of 24 cats and in the liver of four cats in the examinations per-

formed with Real-time PCR from cats whose histopathological findings could not be determined (Figure 2). DNA of *H. heilmannii* could not be detected by Real-time PCR in 4 of 12 cats with gastritis in histopathological examinations. The presence of the DNA of *H. heilmannii* was revealed by Real-time PCR in four cats with hepatitis detected in histopathological examinations.

Figure 2. Amplification curves of 24 cases found positive for H. heilmannii by real-time PCR in the liver tissues of the cats examined in the study. (PC; Positive Control, NC; Negatif Control)

DISCUSSION

There are studies conducted in both human medicine and veterinary medicine to reveal the pathology and zoonosis importance of *Helicobacter* species (Akcakavak et al., 2023; Eaton et al., 1993; Eaton et al., 1996; Happonen et al., 1998; Jalava et al., 1997; Jalava et al., 1998; Diker et al., 2002). In their study on humans, Stolte et al. (1994) determined that 111 of the 125 patients with *H. heilmannii* identified had a history of contact with one or more animals. Stolte et al. (1994), *H. heilmannii* reported that 1.6% of the patients they detected also had *H. pylori*. In studies investigating *H. heilmannii* in cats and dogs, *H. heilmannii* was determined at rates ranging from 20% to 100% (Kubota-Aizawa et al., 2017; Matos et al., 2020) and due to the fact that humans share their habitats with cats and dogs, studies have focused on cats and dogs with the suspicion that these animals may be the source of human diseases with *Helicobacter* species in the aetiology (Eaton et al., 1993; Eaton et al., 1996; Jalava et al., 1997; Jalava et al., 1998; Diker et al., 2002). The fact that DNA *H. heilmannii* was determined by Real-time PCR in 36 of 41 cats examined in this study reveals the importance of studies on the diagnosis and determination of the prevalence of *H. heilmannii* in cats.

Hermanns et al. (1995) stated that they detected Helicobacter-like organisms (HLOs) in 76% of cats in their study determining the histopathological changes in cats and dogs with HLOs. They reported that they detected degeneration in the gastric gland epithelium, neutrophil granulocyte and lymphocyte infiltration, fibrosis and edema in the lamina propria in microscopic examination. Husnik et al. (2022) reported that they detected histopathologically epithelial damage, lymphoid follicular hyperplasia, lymphocytic-plasmacytic cell infiltrations and fibrosis in *Helicobacter* positive in dogs. When the histopathological findings of our study were evaluated, they were compatible with the literature.

Erginsoy et al. (2006) determined Helicobacter-like microorganisms in the gastric mucosa of 28 of the 30 stray cats examined by the immunohistochemical method. Akhtardanesh et al. (2006) used Giemsa staining in cytology examination of stomach samples taken from stray cats in Tehran, and found the infection rate of Helicobacter-like microorganisms in the antrum and stomach body of cases as 63.2% and 77.2%, respectively. The major gastric Helicobacter strains in cats are primarily H. heilmannii and H. felis. According to reports, the prevalence of these two species in cats varies between 57% and 100% (Geyer et al., 1993; Hong et al., 2016). In this study, DNA of 87.8% (36/41) of *H. heilmannii* were detected by Real-time PCR in stomach samples of cats.

In this study, DNA of *H. heilmannii* was identified in 24 cat stomachs in Real-time PCR examinations from cats whose histopathological findings could not be determined, which supports the point of view that *H. heilmannii* may be a part of the stomach flora in cats (Gökalp & Gökalp, 2021). In the prevalence study conducted by Happonen et al (1996), they stated that they found *Helicobacter* spp at equal rates in young and old animals. In the current study, of the 36 cats whose stomach samples were identified as positive for *H. heilmannii* by Real-time PCR, 8 were younger than 1 year old, 16 were between 1-3 years old, and 12 were older than 3 years old. In this study, similar to the results determined in the stomach samples, 6 of the 24 liver samples determined positive for *H. heilmannii* belonged to cats under 1 year old, 10 of them were between 1-3 years old and 8 of them belonged to cats older than 3 years. In the light of the findings of our study, it was thought that *H.*

heilmannii could be encountered more frequently in cats aged 1-3 years and older in the Konya region.

In the study conducted by Sağnak (2007), they reported that they detected *Helicobacter* DNA in 29 (58%) of 50 male dog feces and 36 (78.3%) of 46 female dog feces and the difference between genders was statistically significant. In this study, when the distribution of *H. heilmannii* DNA copies in stomach and liver tissues is examined by gender, it is understood that it is higher in females, similar to the report of Sağnak (2007). However, since the number of samples was insufficient in this study, statistical comparisons could not be made about the relationship of *H. heilmannii* with gender in cats.

Diagnostic methods such as histopathology, cytology, culture, urea breath test and serological tests are often used in human medicine for the diagnosis of *Helicobacter* infections. However, PCR and culture tests are seen as the only methods of identifying agents at the species level (Neiger & Simpson, 2000). In this study, Helicobacter-like microorganisms were detected in 8 cats with HE staining and 19 cats with WS staining, and specific findings could not be detected in the histopathological examinations. All this has strengthened the conviction that Real-time PCR is a rapid, specific and sensitive diagnostic test that can be used to specify *H. heilmannii* in cats.

CONCLUSION

In this study, the presence of *H.heilmannii* in stomach and liver tissues taken during necropsies of domestic, stray and shelter cats that died from different causes was demonstrated by comparing the histopathological findings and Real-time PCR results. As a result, *H. heilmannii* was detected at the rate of 87.8% in cat stomachs by Real-time PCR, and it was found that *H. heilmannii* had a high prevalence in cats in the Konya region of Turkey. In addition, it was concluded that PCR analyzes are very useful in determining the agent, but histopathological examinations are necessary to associate the presence of bacteria with the disease state.

DECLARATIONS

Ethics Approval

It was approved by Selcuk University Veterinary Faculty Experimental Animal Production and Research Center Ethics Committee (02.06.2022, 2022/75).

Conflict of Interest

The authors have no conflicts of interest.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: NT, MT, GA

Data collection and analysis: ZC, AB, RS, VK, MO

Drafting of the manuscript: NT, MT, GA

Critical review: MT, GA

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

Not applicable.

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