Polish Journal of Veterinary Sciences Vol. 19, No. 2 (2016), 237–243

DOI 10.1515/pjvs-2016-0030

Original article

Detection of gastric *Helicobacter* spp. in stool samples of dogs with *gastritis*

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Abstract

The aim of this study was to determine the prevalence and identify the species of gastric *Helicobacter* in the stool of dogs with gastritis. The study was carried out on thirty dogs of different breeds, of both genders and of various ages, diagnosed with gastritis. *Helicobacter* spp. was detected in stool samples using the nested-PCR method. *Helicobacter* bacteria were identified in stool samples from seven (23.3%) dogs. *Helicobacter heilmannii* was found to be the most common species of gastric *Helicobacter*. *Helicobacter salomonis* was identified much less frequently, while *Helicobacter felis*, *Helicobacter pylori* and *Helicobacter bizzozeronii* were not detected in any of the samples.

Key words: *Helicobacter* spp., faeces, dog, PCR

Introduction

Helicobacter bacteria may colonize the gastrointestinal mucosa of humans, domestic animals (such as the dog, cat, pig, ferret and poultry) and wild animals (such as the cheetah or monkey) (Haesebrouck et al. 2009, Casagrande Proietti et al. 2010, Abdi et al. 2014, Hong et al. 2015). In human medicine, *Helicobacter pylori* has been confirmed as contributing to the development of chronic inflammation of the gastric mucosa as well as gastric and duodenal ulcers. In 1994, the International Agency for Research on Cancer (IARC) qualified this bacterium as a class I risk for the development of gastric cancer and gastric mucosa associated lymphoid tissue (MALT) lymphoma (Sjunnesson et al. 2003, Falsafi et al. 2009, Smith et al. 2012). The importance of *Helicobacter* spp. infections in the development of gastric lesions in dogs remains controversial (Shinozaki et al. 2002, Kubiak 2006, Haesebrouck et al. 2009, Jankowski et al. 2015).

Depending on the place of colonization of the gastrointestinal tract, *Helicobacter* spp. is divided into gastric and enterohepatic species. The first group includes species that colonize the upper gastrointestinal tract (stomach and duodenum), such as *Helicobacter pylori*, *Helicobacter heilmannii*, *Helicobacter felis*, *Helicobacter salomonis*, *Helicobacter bizzozeronii*, *Helicobacter suis* and *Helicobacter mustelae*. The second group contains species found in the lower gastrointestinal tract (ileum, colon, rectum and liver – bile ducts), such as *Helicobacter canis*, *Helicobacter bilis*, *Helicobacter rappini*, *Helicobacter hepaticus*, *Heli-*

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cobacter fennelliae, *Helicobacter cinaedi* and *Helicobacter pullorum* (Shinozaki et al. 2002, Kubiak 2006, Haesebrouck et al. 2009, Ekman et al. 2013, Abdi et al. 2014).

Currently, in veterinary medicine invasive methods are used to detect *Helicobacter* spp., involving gastroscopy and collecting a biopsy sample of the gastric mucosa in order to carry out a rapid urea test (RUT), a histopathological examination, direct Gram stains, a microbiological culture and electron microscopy. Bacterial DNA is detected using PCR. These methods have a high specificity and sensitivity. However, they also have a number of drawbacks. These include patient discomfort associated with carrying out a gastroscopy, obtaining biopsy samples and general anaesthesia associated with these procedures (Shinozaki et al. 2002, Kubiak 2006, Haesebrouck et al. 2009, Hong et al. 2015).

Given the difficulties in culturing animal gastric *Helicobacter* spp. and the fact that not all veterinary professionals are able to perform a gastroscopy with a biopsy of the gastric mucosa, other non-invasive methods are sought in veterinary medicine to detect these bacteria in saliva and stool samples.

The aim of the study was to determine the prevalence and identify the species of gastric *Helicobacter* in the stool of dogs with gastritis.

Materials and Methods

The study was carried out on 30 dogs with gastritis, of different breeds and age and of both genders. Gastritis was diagnosed based on the clinical signs (dyspeptic symptoms), macroscopic gastric mucosa lesions detected during gastroscopy, and histopathological changes seen in the biopsy samples, which were evaluated according to the Sydney classification.

Rectal swab specimens were used for the PCR study. Once collected, they were placed in a container and frozen at -20°C.

Isolation of DNA from the stool

A large amount of polymerase-inhibiting compounds may cause false-negative PCR results. In order to eliminate this phenomenon, an Omega Bio-tek Inc. „Stool DNA kit" (cat. No. D4015-01) was used, which contained polymerase inhibitors. Reversible interactions between the DNA and the silica bed were used in the kit.

The thawed 200 g stool sample was placed in an Eppendorf tube containing 200 mg of glass beads. 540 µl of a Spheroid Lysis Buffer (SLB) was then added. The stool sample was subsequently homogenised. 60 µl of DS buffer and 20 µl of proteinase K were added to the sample, which was then incubated at 70°C. 10 minutes later, 200 µl of an SP2 buffer was added, and the mixture was centrifuged for 5 minutes (13 000 x g). 400 µl of the supernatant was then placed in a clean tube. 200 µl of the HTR buffer was added and the tube was centrifuged for 2 minutes (13 000 x g). Following centrifugation, 250 µl of the supernatant was poured into a clean tube. 250 µl of the lysis buffer (BL) and 250 µl of ethanol were added to this tube. This sample was centrifuged, placed on a column and centrifuged once more for 1 minute. It was then washed with a 500 µl VHB buffer and 700 µl wash buffer. The membrane of the column was dried and saturated with 10 mM Tris-HCl, (pH 8.5) and centrifuged. The retrieved DNA was suspended in 100 µl 10 mM Tris-HCl buffer, (pH 8.5) for further use in the nested-PCR.

Extraction of DNA from gastric mucosa specimens

An Omega Bio-tek, Inc. „Tissue DNA kit" (nr kat. D3396-01) was used to extract the DNA from the gastric mucosa. The tissue sections were thawed and subjected to protease digestion at 55°C for 3 hours. The cells were then placed in a lysis buffer (BL) with detergent at 70°C. The cell lysate was then mounted on a silica gel column which selectively bound DNA at a pH lower than 7.5. The column was washed with 500 µl of HB buffer and 700 µl of washing buffer. After the membrane was dried, the column was saturated with 10 mM of Tris-HCl at pH 8.5 and was centrifuged. A pH change led to DNA extraction. DNA could then be used in further stages of the analysis.

Nested-PCR

This method was used to detect *Helicobacter* microorganisms and to identify their species. It consists of performing two subsequent PCR reactions. In the first reaction, the DNA retrieved from samples is used as the matrix, and a pair of external F and R primers is applied. The DNA amplification conditions for the first nested-PCR reaction are shown in Table 1. Together with polymerase and a pair of WF and WR primers, the product of the first reaction forms the matrix of the second reaction. The DNA amplification conditions for the second nested nested-PCR reaction are shown in Table 2. This increases the sensitivity of the method. At the same time, the

Table 1. DNA amplification conditions in the first nested-PCR reaction for various *Helicobacter* species.

Stages of first nested – PCR reaction	*Helicobacter heilmannii*	*Helicobacter salomonis*	*Helicobacter bizzozeronii*	*Helicobacter felis*	*Helicobacter pylori*
Initial denaturation	temp. – 94°C, time – 3 min	temp. – 95°C, time – 5 min	temp. – 95°C, time – 5 min	temp. – 95°C, time – 5 min	temp. – 95°C, time – 5 min
Initial primers connection	temp. – 57°C, time – 2 min	–	–	–	–
Initial elongation	temp. – 72°C, time – 3 min	–	–	–	–
Number of cycles	4	–	–	–	–
Appropriate denaturation	temp. – 94°C, time – 30 s	temp. – 94°C, time – 30 s	temp. – 94°C, time – 1 min	temp. – 94°C, time – 1 min	temp. – 94°C, time – 45 s
Primers connection	temp. – 57°C, time – 30 s	temp. – 55°C, time – 30 s	temp. – 57°C, time – 1 min	temp. – 52°C, time – 1 min	temp. – 50°C, time – 45 s
Appropriate elongation	temp. – 72°C, time – 1 min	temp. – 72°C, time – 1 min	temp. – 72°C, time – 1 min	temp. – 72°C, time – 1 min	temp. – 72°C, time – 3 min
Number of cycles	31	30	35	28	24
Final elongation	temp. – 72°C, time – 5 min	temp. – 72°C, time – 10 min	temp. – 72°C, time – 10 min	temp. – 72°C, time – 7 min	temp. – 72°C, time – 5 min
End of reaction	temp. – 4°C (until removal of the sample from the thermocycler)	temp. – 4°C (until removal of the sample from the thermocycler)	temp. – 4°C (until removal of the sample from the thermocycler)	temp. – 4°C (until removal of the sample from the thermocycler)	temp. – 4°C (until removal of the sample from the thermocycler)

Table 2. DNA amplification conditions in the second nested-PCR reaction for various *Helicobacter* species.

Stages of second nested – PCR reaction	*Helicobacter heilmannii*	*Helicobacter salomonis*	*Helicobacter bizzozeronii*	*Helicobacter felis*	*Helicobacter pylori*
Initial denaturation	temp. – 95°C, time – 5 min	temp. – 95°C, time – 5 min	temp. – 95°C, time – 5 min	temp. – 95°C, time – 5 min	temp. – 95°C, time – 5 min
Appropriate denaturation	temp. – 94°C, time – 3 min	temp. – 94°C, time – 30 s	temp. – 94°C, time – 1 min	temp. – 94°C, time – 3 min	temp. – 94°C, time – 45 s
Primers connection	temp. – 58,5°C, time – 45 s	temp. – 62°C, time – 30 s	temp. – 60°C, time – 45 s	temp. – 57°C, time – 45 s	temp. – 59°C, time – 45 s
Appropriate elongation	temp. – 72°C, time – 1 min	temp. – 72°C, time – 1 min	temp. – 72°C, time – 1 min	temp. – 72°C, time – 1 min	temp. – 72°C, time – 45 s
Number of cycles	35	30	35	35	34
Final elongation	temp. – 72°C, time – 5 min	temp. – 72°C, time – 10 min	temp. – 72°C, time – 10 min	temp. – 72°C, time – 5 min	temp. – 72°C, time – 5 min
End of reaction	temp. – 4°C (until removal of the sample from the thermocycler)	temp. – 4°C (until removal of the sample from the thermocycler)	temp. – 4°C (until removal of the sample from the thermocycler)	temp. – 4°C (until removal of the sample from the thermocycler)	temp. – 4°C (until removal of the sample from the thermocycler)

use of two different sets of primers ensures a high specificity of the method and eliminates false-positive results. The use of the primers and PCR schemes are presented in Table 3.

Thermo Scientific™ DreamTaq DNA Polymerase (catalogue number EP0703) was used to synthetize DNA.

The validation of the PCR reaction was carried out using the following reference strains: *Helicobacter pylor* (strain ATCC 700392/26695), *Helicobacter felis* (strain ATCC 49179), *Helicobacter bizzozeronii* (strain CCUG 35545), *Helicobacter salomonis* (strain CCUG 37845) and *Helicobacter heilmannii* (a strain cultured by the Department of Microbiology of the Faculty of Medicine of the Wroclaw Medical University).

Table 3. Summary of the primers and PCR reactions used in the study.

Gene detected	Species detected	Type of PCR reaction	Stage of PCR process	Primers	Sequence of primer nucleotides in 5'-3' orientation	PCR product
ure*A*	*H. pylori* M60398	nested	first	PylF PylR	CCA GAT GAT GTG ATG GAT GG TCA AGT CTG TAT CGC CCA ATC	607
			second	HPU1 HPU2	GCCAATGGTAAATTAGTT CTCCTTAATTGTTTTTAC	411
ureB	*H. heilmannii* L25079	nested	first	HeilF HeilR	GGGCGATAAAGTGCGCTTG CTGGTCAATGAGAGCAGG	580
			second	WheilF WheilR	GGCATTTACAAAGCCGACAT ACCAAGGTAGCCAAGGTTCA	354
ureB HSP60	*H. Felis* X69080	nested	first	FelisF FelisR	ATGAAACTAACGCCTAAAGAACTAG GGAGAGATAAAGTGAATATGCGT	1150
			second	Fe1F Fe3R	TTT GGT GCT CAC TAA CGC CCT C TTC AAT CTG ATC GCG TAA AG	434
	H. bizzozeronii AJ130881	nested	first	BizzF BizzR	GAA GTC GAA CAT GAC TGC AC GGT CGC ATT AGT CCC ATC AG	420
			second	Bi1F Bi2R	AAC CAA YAG CCC CAG CAG CC TGG TTT TAA GGT TCC AGC GC	373
	H. Salomonie AJ558226	nested	first	HSALF HSALR	CATTTTCAAAGAGGGCTTGC GCACACCCCTCAGTTTGTTT	518
			second	WSALF WSALR	TGGAGCTAATCCCATTGAGG CTAAGGTTGTGAGGGCTTCG	461

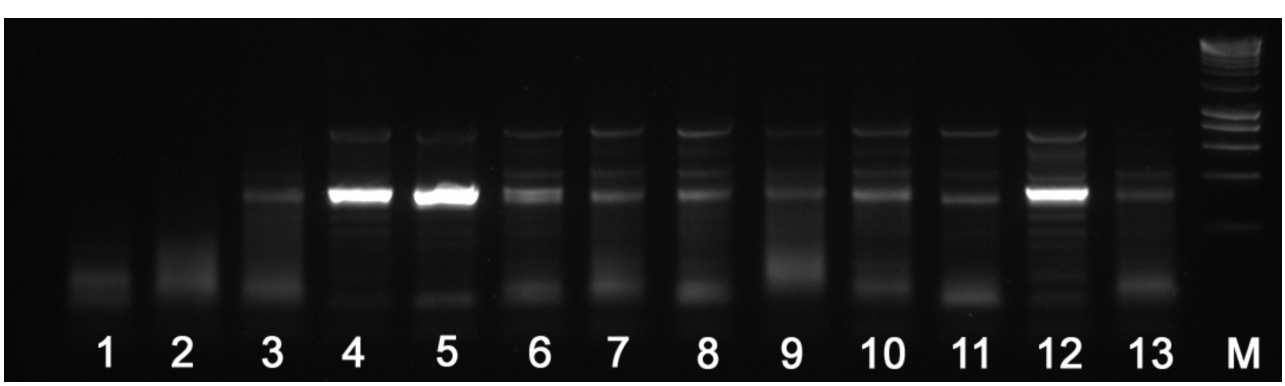

Fig. 1. Gel electrophoresis of nested-PCR products using primers for *ureB* gene from *Helicobacter heilmannii* (primers WheilF and WheilR), specific product (lines 4, 5, 12) is 354 bp

Results

Results of stool PCR

Based on the nested-PCR molecular study, the presence of gastric *Helicobacter* microorganisms was found in seven (23.3%) dogs. In the remaining 23 dogs (76.7%), there were no gastric *Helicobacter* spp. In all the samples, there was a single *Helicobacter* species present. The most commonly occurring species of gastric *Helicobacter* spp. in faeces was *Helicobacter heilmannii* (Fig. 1.), which was found in five (71.4%) cases. *Helicobacter salomonis* was identified in two (28.6%) cases. *Helicobacter felis*, *Helicobacter pylori* and *Helicobacter bizzozeronii* were not found in any of the samples.

Results of gastric sample PCR

Helicobacter bacteria were detected in all the dogs in the sections of the gastric mucosa using nested-PCR. An infection with a single *Helicobacter* species (*Helicobacter heilmannii)* was found in 30% of

the cases. 50% of the dogs were infected with two species in the following combinations: *Helicobacter heilmannii* + *Helicobacter bizzozeronii* (six cases), *Helicobacter heilmannii* + *Helicobacter salomonis* (five cases), *Helicobacter heilmannii* + *Helicobacter felis* (one case) i *Helicobacter felis* + *Helicobacter salominis* (one case). 16.7% of the dogs were infected with three *Helicobacter* species. The combinations included *Helicobacter heilmannii* + *Helicobacter bizzozeronii* + *Helicobacter salomonis* infections (four cases) and a *Helicobacter heilmannii* + *Helicobacter bizzozeronii* + *Helicobacter felis* infection (one case). 3.3% of the dogs were infected with *Helicobacter heilmannii* + *Helicobacter bizzozeronii* + *Helicobacter felis* + *Helicobacter salominis*. The most frequently detected species in the sections of the gastric mucosa was *Helicobacter heilmanii* (present in 96.7% of the dogs), whereas *Helicobacter bizzozeronii* was detected in 40% of the dogs, *Helicobacter salomonis* was identified in 36.7% of the dogs, *Helicobacter felis* was present in 13.3% of the cases and *Helicobacter pylori* was found in 6.7% of the cases.

Discussion

One of the non-invasive methods used to diagnose Helicobacter *pylori* infection in people is faecal PCR. This method is rarely used in veterinary medicine, and there are few reports describing this method in veterinary literature. Therefore, it is difficult to determine the incidence of gastric *Helicobacter* spp. in canine faecal samples. In this study, using nested-PCR to assess stool samples, *Helicobacter* microorganisms were identified in 23% of animals. Hong et al. (2015) obtained a higher percentage of gastric *Helicobacter* spp. DNA in faeces, amounting to 62.5%. However, such a large percentage of *Helicobacter* spp. may have been due to the small study sample (8 dogs). Using PCR to detect *Helicobacter* spp. DNA in stool, Ekman et al. (2013) detected entero-hepatic *Helicobacter canis* (14 cases) and *Helicobacter bilis* (2 cases), but did not find gastric *Helicobacter* spp. in any of the faecal samples. Casagrande Proietti et al. (2010) detected the presence of entero-hepatic *Helicobacter* species in pig stool using PCR in 60% of the studied samples. None of these samples contained gastric *Helicobacter* spp., such as *Helicobacter suis* or *Helicobacter pylori,* reported to occur in pigs. In humans, *Helicobacter pylori* DNA is detected in 25% to 100% of stool samples. The large discrepancy in results in human and veterinary medicine may result from the small amount of gastric *Helicobacter* spp. in faeces, a degradation of bacterial DNA in the large intestine, the presence of polymerase inhibitors such as complex polysaccharides, and may depend on the DNA extraction method and type of PCR used (Kabir 2001, Shinozaki et al. 2002, Falsafi et al. 2009, Smith et al. 2012, Hong et al. 2015).

We found that gastric *Helicobacter* spp. were detected 77% more often in sections of the gastric mucosa than in the stool samples. The low incidence of these bacteria in stool was confirmed by Ekman et al. 2013, who did not find any gastric *Helicobacter* spp but did find enterohepatic *Helicobacter* species in stool samples. On the other hand, Hong et al. 2015, who identified gastric *Helicobacter* spp. DNA in the stool and sections of the gastric mucosa, indicated that 100% of the species found at the two sites concurred. However, that study was limited by the small sample size.

Helicobacter heilmannii, *Helicobacter felis*, *Helicobacter salomonis*, *Helicobacter bizzozeronii* and *Helicobacter pylori* have been found to colonize the stomach in dogs (Kubiak 2006, Haesebrouck et al. 2009, Ekman et al. 2013, Abdi et al. 2014). The prevalence of these species varies with the geographical location. We found *Helicobacter heilmannii* to be the most common gastric *Helicobacter* species, which was present in 71% of the faecal samples. *Helicobacter salomonis* was detected much less frequently (28.6%). In their study, Hong et al. (2015) reported that *Helicobacter heilmannii* (37.5% of cases) was detected most commonly in their study group. They also detected *Helicobacter felis* (25% of cases) in the samples. A high incidence of *Helicobacter heilmannii* in dogs in Poland was confirmed by Kubiak (2006), who carried out a study on 20 healthy and 137 sick dogs and found *Helicobacter heilmannii* in 95% and 83.2% of the dogs, respectively. The authors of this study carried out a study on 49 dogs with gastric ulcers and found *Helicobacter heilmannii* to be the most common species (73.9% of cases) (Jankowski et al. 2015).

A *Helicobacter* spp. infection can be diagnosed using stool samples based on a microbiological culture, the determination of anti-*Helicobacter* antibodies, and bacterial DNA using PCR (Lehmann and Beglinger 2003, Mishra et al. 2008, Falsafi et al. 2009, Smith et al. 2012). Due to the low sensitivity of microbiological stool culture this method is rarely performed. Moreover, *Helicobacter pylori* can be difficult to culture. Likewise, gastric Helicobacter species present in dogs rarely grow on a growth medium, which has been confirmed in numerous studies (Andersen et al. 1996, Shinozaki et al. 2002, Sjunnesson i wsp. 2003, Kubiak 2006, Mishra et al. 2008). Some other factors which may limit the culture of these bacteria from stool samples are: a high concentration of other microorganisms present in faeces, a low concentration of gastric *Helicobacter* spp., the long passage of the bacteria

through the gastrointestinal tract, and the presence of granulomatous bacteria and dead bacteria in faeces (Kabir 2001, Lehmann and Beglinger 2003, Falsafi et al. 2009, Smith et al. 2012). Hence, microbiological cultures are not performed routinely to diagnose *Helicobacter* spp. infections (Lehmann and Beglinger 2003, Mégraud and Lehours 2007). Another method, which detects *Helicobacter* spp. infection, is the identification of *Helicobacter pylori* antigens in stool. This method determines monoclonal antibodies directed against Helicobacter *pylori* antigens present in the faeces. The sensitivity and specificity of this method is estimated to be between 58% – 96% and 67% – 100%, respectively. *Helicobacter pylori* may be degraded during intestinal passage and by mucolytic drugs, which reduces the effectiveness of this method (Kabir 2001, Cirak et al. 2007, Mishra et al. 2008, Tonkić et al. 2012, Garza-Gonzalez et al. 2014). Since the method uses monoclonal antibodies directed against *Helicobacter pylori* antigens, it is of limited use in dogs, since they tend to suffer from Helicobacter *heilmannii*, *Helicobacter felis*, *Helicobacter salomonis,* and *Helicobacter bizzozeronii* infections. The polymerase chain reaction (PCR) may be used to detect Helicobacter spp. in faecal samples. The sensitivity and specificity of this method are between 69% – 94% and 97.1% – 100%, respectively. Polymerase inhibitors present in stool may limit the effectiveness of this method. On the other hand, the test does not require a high concentration of bacteria and/or the presence of live bacteria. The use of a semi-nested or nested-PCR increases the accuracy of this method compared to a classical PCR (Lehmann and Beglinger 2003, Mishra et al. 2008, Smith et al. 2012, Tonkić et al. 2012). Hong et al. (2015) concluded that faecal PCR is a useful technique in the detection of Helicobacter spp. infections in dogs.

Despite years of research, the routes of *Helicobacter* spp. infection remain unknown. The transmission of *Helicobacter* spp. can be direct, i.e. oral-oral, faecal-oral or gastro-oral as well as indirect, through contaminated food, water and poorly disinfected endoscopic equipment (Brown 2000, van Duynhoven and de Jonge 2001, Kusters et al. 2006, Khalifa et al. 2010). Due to the high incidence of *Helicobacter* spp. in the stomachs of animals, they are considered to be a potential risk factor in transmitting the infection to humans (Kusters et al. 2006, Khalifa et al. 2010, Abdi et al. 2014). It is thought that the faecal-oral mode of transmission occurs less frequently than oral-oral transmission. This may be due to the fact that *Helicobacter* spp. are susceptible to the bactericidal effect of bile. The detection of *Helicobacter* bacteria DNA using PCR in faeces does not confirm the presence of pathogens capable of infection. The latter may be cultured from faeces. However, this is immensely difficult. *Helicobacter pylori* was successfully cultured in one adult and seven children in Gambia and 12 adults in the United Kingdom (Brown 2000, Khalifa et al. 2010). Ekman et al. (2013) argue that it is highly unlikely for canine gastric *Helicobacter* spp. to be transmitted via the faecal-oral route. Contrary to this opinion, Hong et al. (2012) claim that since gastric Helicobacter spp. are present in faeces, this must also be their habitat. The study of Abdi et al. (2014), who found *Helicobacter bizzozeroni* and *Helicobacter salomonis* in 100% and 80% of dogs, respectively, confirms this. The authors of the current study have made similar observations and found *Helicobacter heilmannii* and *Helicobacter salomonis* in seven animals.

This study indicates that *Helicobacter* spp. rarely occurs in stool samples of dogs with gastritis, and the most commonly identified species of this microorganism is *Helicobacter heilmannii*. The results may indicate that canine faeces containing *Helicobacter* spp. can be a potential source of contamination of water and food and infection of humans and other animals (gastric *Helicobacter* spp. may cause gastro-intestinal disorders). This, however, warrants further studies.

Acknowledgements

Article publication supported by Wrocław Centre of Biotechnology, programme the Leading Notional Research Centre (KNOW) for years 2014-2018.

References

Abdi FS, Jamshidi S, Moosakhani F, Sasani F (**2014**) Detection of *Helicobacter* spp. DNA in the colonic biopsies of stray dogs: molecular and histopathological investigation. Diagn Pathol 9: 50.

Andersen LP, Norgaard A, Holck S, Blom J, Elsborg L (**1996**) Isolation of a „Helicobacter heilmanii" – like organism from the human stomach. Eur J Clin Microbiol Infect Dis 15: 95-96.

Brown LM (**2000**) *Helicobacter pylori*: Epidemiology and Routes of Transmission. Epidemiol Rev 22: 283-297.

Casagrande Proietti P, Bietta A, Brachelente C, Lepri E, Davidson I, Franciosini MP (**2010**) Detection of *Helicobacter* spp. in gastric, fecal, and saliva samples from swine affected by gastric ulceration. J Vet Sci 11: 221-225.

Cirak MY, Akyon Y, Megraud F (**2007**) Diagnosis of *Helicobacter pylori*. Helicobacter 12 (Suppl 1): 4-9.

Ekman E, Fredriksson M, Trowald-Wigh G (**2013**) Helicobacter spp. in the saliva, stomach, duodenum and faeces of colony dogs. Vet J 195: 127-129.

Falsafi T, Favaedi R, Mahjoub F, Najafi M (**2009**) Application of Stool-PCR test for diagnosis *Helicobacter pylori* infection in children. World J Gastroenterol 15: 484-488.

Garza-Gonzhlez E, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla FJ (**2014**) A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication. World J Gastroenterol 20: 1438-1449.

Haesebrouck F, Pasmans F, Flahou B, Chiers K, Baele M, Meyns T, Decostere A, Ducatelle R (**2009**) Gastric Helicobacters in Domestic Animals and Nonhuman Primates and Their Significance for Human Health Clin Microbiol Rev 22: 202-223.

Hong S, Chung Y, Kang WG, Choi YS, Kim O (**2015**) Comparison of three diagnostic assays for the identification of *Helicobacter spp.* in laboratory dogs. Lab Anim Res 31: 86-92.

Jankowski M, Spużak J, Kubiak K, Glińska-Suchocka K, Biernat M, Kiełbowicz Z (**2015**) Risk Factors of Gastric Ulcers in Dogs. Pak Vet J 35: 93-97.

Kabir S (**2001**) Detection of *Helicobacter pylori* in faeces by culture, PCR and enzyme immunoassay. J Med Microbiol 50: 1021-1029.

Khalifa MM, Sharaf RR, Aziz RK (**2010**) *Helicobacter pylori*: a poor man's gut pathogen? Gut Pathog 2: 2.

Kubiak K (**2006**) Kolonizacja błony śluzowej żołądka psów i kotów drobnoustrojami z rodzaju *Helicobacter* – aspekt kliniczny. 1st ed., Zeszyty Naukowe Akademii Rolniczej we Wrocławiu, Wrocław.

Kusters JG, van Vliet AH, Kuipers EJ (**2006**) Pathogenesis of *Helicobacter pylori* Infection Clin Microbiol Rev 19: 449-490.

Lehmann FS, Beglinger C (**2003**) Current Role of *Helicobacter pylori* Stool Tests. Digestion 68: 119-123.

Megraud F, Lehours P (**2007**) *Helicobacter pylori* Detection and Antimicrobial Susceptibility Testing. Clin Microbiol Rev 20: 280-322.

Mishra S, Singh V, Rao GR, Jain AK, Dixit VK, Gulati AK, Nath G (**2008**) Detection of *Helicobacter pylori* in stool specimens: comparative evaluation of nested PCR and antigen detection. J Infect Dev Ctries 2: 206-210.

Sjunnesson H, Fält T, Sturegård E, Abu Al-Soud W, Ljungh A, Wadström T (**2003**) PCR-Denaturing Gradient Gel Electrophoresis and Two Feces Antigen Tests for Detection of Helicobacter pylori in Mice. Curr Microbiol 47: 278-285.

Smith SI, Fowora MA, Lesi OA, Agbebaku E, Odeigah P, Abdulkareem FB, Onyekwere CA, Agomo CA, Contreras M (**2012**) Application of stool-PCR for the diagnosis of *Helicobacter pylori* from stool in Nigeria – a pilot study. Springerplus 1: 78.

Shinozaki JK, Sellon RK, Cantor GH, Besser TE, Mealey KL, Vaden SL (**2002**) Fecal Polymerase Chain Reaction with 16S Ribosomal RNA Primers Can Detect the Presence of Gastrointestinal Helicobacter in Dogs. J Vet Intern Med 16: 426-432.

Tonkić A, Tonkić M, Lehours P, Megraud F (**2012**) Epidemiology and Diagnosis of *Helicobacter pylori* Infection. Helicobacter 17 Suppl. 1: 1-8.

van Duynhoven YT, de Jonge R (**2001**) Transmission of *Helicobacter pylori*: a role for food? Bull World Health Organ 79: 455-460.