j.Egypt.vet.med.Assoc 79, no 4. 963 – 972 (2019)

DETECTION OF *HELICOBACTER* SPECIES IN COMPANION ANIMALS REGARDING TO ITS ZOONOTIC RISK

By

Mariam Beder[1], Sherif Marouf[1], Kareem Abdelaziz[2] , Jakeen Eljakee[1]

[1]Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.

[2]Tropical Medicine Department, Faculty of Medicine, Ain shams university, Cairo, Egypt.

ABSTRACT

Helicobacteriosis is worldwide infection caused by *Helicobacter* species. It affected both human and animals. The study investigate *Helicobacter* species in companion animals (Dogs and Cats) and its zoonotic and public health repertoire. Samples were collected from apparently healthy companion animals (70 dogs and 65 cats) and 70 human patients in contact with the companion animal from Cairo and Giza governorates. The samples included serum samples, feaces and stool samples and biopsies of gastric fundus fragments 5 mm approximately. All samples examined by culture, biochemical, serology and molecular identification. The study reveals 43.4% *Helicobacter* species by PCR. *H. heilmannii* is more predominant with ratio 16% and *H.pylori* reveals 6%, while all samples were negative for *H. felis*. In conclusion, dogs and cats are reservoir and play a major transmission route for human helicobacters infection.

Key words:

Helicobacteriosis, *H. heilmannii*, *H. pylori*, *H. felis*, gastric ulcer.

INTRODUCTION

The bacteria were first named *Spirillum and has been* found in the gastric mucosa of a dog [1] and his work was confirmed by Bizzozero and Salomon in dogs, cats, and rats [2,3]. Then were named *Spirochete* then they were classified *Campylobacter*, and now they belong to the genus *Helicobacter* [4,5]. The genus *Helicobacter* presently includes approximately 21 species, and individual species can have a gastric, intestinal, or hepatic distribution. *Helicobacter* species associated with gastric infection include *H. pylori* in humans and *H. felis, H. bizzozeronii, H. salomonis,* ‘‘*H. heilmannii,*’’ ‘‘*Flexispira rappini,*’’ and *H. bilis* in dogs and cats. *H. heilmannii* was formerly known as *Gastrospirillum hominis,* and organisms once considered ‘‘*F. rappini*’’ are now known to be *Helicobacter* species.8 *Helicobacter* species

associated with intestinal colonization include *H. fennelliae, H. cinaedi, H. canis,* and ‘‘ *F. rappini*’’ in humans, dogs and cats and *H. hepaticus, H. bilis, H rodentium, H. muridarum,* and ‘‘*F. rappini*’’ in mice.4 Hepatic *Helicobacter* species include *H. canis* in dogs, *H. hepaticus* and *H. bilis* in mice, and possibly *H. pylori* in humans.[6]

Prevalence studies have suggested that gastric *Helicobacter* infections are almost universally present in both apparently healthy dogs and dogs presenting with clinical gastric disease[7]. However, Prevalence of canine and feline helicobacters is high. Prevalence rates of 86 to 100 % in healthy dogs, 61 to 82% in dogs with upper gastrointestinal signs, 41 to100 % in healthy cats, and 56 to76% in affected cats have been reported. *H.pylori* infection is a common, usually lifelong, infection that is found worldwide. Studies suggest that infection rates vary according to geographic region[8].The correlation of *Helicobacter* spp. in the pathogeny of gastritis and gastric ulcer has been demonstrated, and more recently the bacteria has been identified as the inducing agent of gastric carcinoma in humans[9]. Studies comprising the histological evaluation of cats and dogs' stomach revealed the presence of the bacterium as a predominant occurrence in the body and gastric fundus.However, the degree of colonization by those bacteria does not correlate directly with the diagnosis of mild to moderate gastritis in cats and dogs[10].

Evidence is accumulating that especially pigs, dogs, and cats constitute reservoir hosts for gastric *Helicobacter* species with zoonotic potential. Many studies reporting *H. pylori* in commercial vendor cats led to a suggestion that *H. pylori* may be a zoonotic pathogen with transmission occurring from cats to humans [11]. Evidence suggests the potential of animals, especially domestic ones, to be a source of zoonotic infection by helicobacteria, since bacteria with similar morphology to that found in animals were observed in the stomach of humans with gastritis [12]. This fact deserves close attention because most of the world population presents direct contact with a domestic animal species, mainly dogs. However, the exact way the transmission of this microorganism occurs remains unknown.The isolationof *Helicobacter* spp. from saliva, dental plaque, and feces of dogs reinforces the hypothesis of transmission by these animals, oro-oral or oro-fecal via [13].Not all *Helicobacter* species are readily culturable,[14] and multiple techniques requiring cultured organisms may be needed for accurate determination of species of *Helicobacter.* Serologic tests for helicobacteriosis are not yet clinically available for veterinary application. Detection of fecal *H. pylori* antigen is possible. A polymerase chain reaction (PCR) assay is a simple,noninvasive, sensitive, and specific

diagnostic test that allow the documentation of *Helicobacter* infection in human, dogs and cats. Thepresent investigation aimed atevaluating the prevalence of *Helicobacter* spp.in companion animals and to correlate their transmission role as a zoonotic risk [15].

MATERIAL AND METHODS

Animal ethics:

This study was carried out according to the principles of the Declaration of Egypt and approved from Veterinary Medicine Cairo University Institutional Animal Care and Use Committee Ref; VETCU1022109045.

Human ethics:

The study had full ethical approval from Faculty of medicine Ethics Committee according to the principles of the Declaration of Egypt.

Samples:

Samples were collected from apparently healthy companion animals from Cairo and Giza governorates. They included 70 dogs (30 serum samples, 30 feaces samples and 10 biopsies of gastric fundus fragments 5 mm approximately), 65 cats (30 serum samples, 30 feaces samples and 5 biopsies of gastric fundus fragments 3 mm approximately).

Seventy samples were collected from human patients in contact with companion animals under study and suffering from dyspepsia, chronic vomiting, and perforated peptic ulcer (30 serum samples, 30 stool samples and 10 biopsies of gastric fundus fragments approximately 5 mm.

All samples were derived during the period from March, 2016 to March 2019 and examined for the presence of *Helicobacter* species.

Isolation and identification of *Helicobacter* species:

Five grams of feaces, stool and biopsies samples were bacteriologically homogenized separately according to Forbes *et al* [16] with 15 ml Brucella broth (Sigma, USA) containing 20% glycerol (Sigma-Aldrich, USA) and 0.5 g cholestyramine (Sigma, USA). A loop-full was streaked onto Columbia blood agar plates (Oxoid) supplemented with Helicobacter selective supplement (SR0147E, Oxoid). All plates were incubated under microaerophilic conditions at 37 °C for 3 to 5 days. Purified colonies were through were gram staining for microscopic examination then biotyping based on Catalase production, Oxidase production, Urea hydrolysis, Nitrate reduction and Salt tolerance.

2.5. Serological detection of *Helicobacter pylori*:

Feaces and stool samples were serologically detected for presence of *H. pylori* antigen using Asan Easy Test *H. pylori* Ag (REF: 24111, Korea); while serum samples were applied for *H. pylori* antibodies using Asan Easy Test *H. pylori* Ab (REF: 14131, Korea). The procedure and result reading were applied according the manufacturing.

2.6. Molecular identification:

Fecal, stool and biopsies DNAs were extracted from using QIAamp mini kit (Catalogue no.51304, Qiagen, Switzerland), according to the manufacturer's instructions with some modifications. Primers were utilized in a 25- µl reaction containing 6 µl of template, 1 µl of each primer of 20 pmol concentrations, 12.5 µl of Emerald Amp Max PCR Master Mix (Emerland, Japan) and 4.5 µl of DEPC water. The reaction was performed in a Biometra thermal cycler (Germany). The sequence and thermal profile for each primer is listed in Table (1) [17,18,19]. The products of PCR were separated by electrophoresis according to **Sambrook *et al.*** [20]. The gel was photographed by a gel documentation system (Biometra BDA digital, Germany).

Table (1): Primer sequencing, amplified product and cycling conditions of the different primers used in Helicobacter species.

Table (1):

Gene	Sequence	Amplified product	Cycling conditions of the different primers					
			Primary denaturation	Secondary denaturation	Annealing	Extension	Cycles No.	Final extension
H. felis urea, ureB	GTG AAG CGA CTA AAG ATA AAC AAT	241 bp	94°C/ 5 min.	94°C/ 30 sec.	62°C/ 30 sec.	72°C/ 30 sec.	35	72°C/ 7 min.
	GCA CCA AAT CTA ATT CAT AAG AGC							
H. heilmannii ureB	GGG CGA TAA AGT GCG CTT G	580 bp			58°C/ 40 sec.	72°C/ 45 sec.		72°C/ 10 min.
	CTG GTC AAT GAG AGC AGG							
H. pylori glmM	GGA TAA GCT TTT AGG GGT GTT AGG GG	296 bp			57°C/ 40 sec.	72°C/ 45 sec.		72°C/ 10 min.
	GCT TAC TTT CTA ACA CTA ACG CGC							
Helicobacter spp. 16S rRNA	AAG GAT GAA GCT TCT AGC TTG CTA	398 bp			50°C/ 40 sec.	72°C/ 40 sec.		72°C/ 10 min.
	GTG CTT ATT CGT GAG ATA CCG TCA T							

RESULTS

Microbiological results:

Twenty samples out of 115 faeces stool and biopsies samples reveals round, small and translucent colonies at culture with incidence 17.4%. Gram staining show gram negative, Spiral, helical or curved with blunt ends, non-spore former microorganism. Biochemical profile is catalase positive,oxidase positive, urea hydrolysis positive, and negative nitrate reduction and tolerate 1.25% NaCl.The distribution of positive culture is high prevalent in dogs 22.5% (9/40; 6 from feaces and 3 from gastric biopsies). The incidence in cat is 14.3% (5/35; 4 from feaces and one fundus biopsy), but the rate in human reveals 15% (6/40; 3 from stool and 3 fundus biopsy).

Serological result of *Helicobacter pylori*:

Seventy serum samples have positive antibody reaction at using Asan Easy Test *H. pylori* Ab. The dog serum reveals high prevalence (100 %) for *H. pylori*, while human and cat serum positivity is 83% (25/30) and 50% (15/30) respectively. A total of 58 *H. pylori* Ag are detecting in feaces and stool samples by Asan Easy Test *H. pylori* Ag. The rates of *H. pylori* Ag are 93% (28/30), 73% (22/30) and 26.6% (8/30) for dog feaces, human stool and cat feaces respectively.

Prevalence of *Helicobacter* species based on PCR:

Fifty samples out of 115 samples (43.4%) reveal amplification band of 398 bp by PCR based on 16S rRNA primer that means *Helicobacter* species Fig. (1).

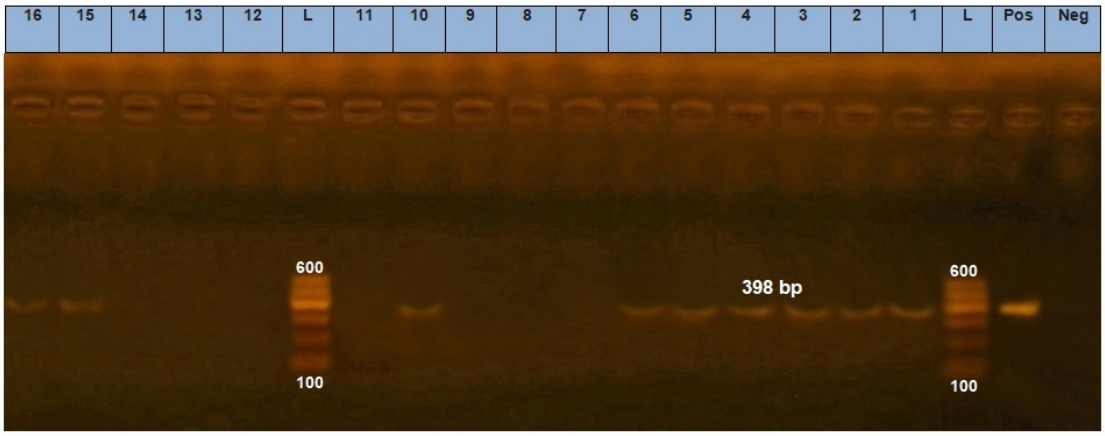

Fig. (1): PCR amplification from dogs, cats feces and gastric biopsies as well as human stool and gastric biopsies using 16S rRNA primer. The expected size of the product is 398 bp. Lanes: L, 100-600bp DNA ladder; Neg, reagent control (no DNA); Pos, DNA extracted from a known positive dog feaces sample; 1- 4, DNA extracted from dog feaces and gastric biopsy;5, 6, DNA

extracted from cat feaces and gastric biopsy and 10,15,16, DNA extracted from human stool and gastric biopsy.

At the level of the species based on primers in (Table 1), *H. heilmannii* is more predominant (amplification fragments at 580 bp) with ratio 16% (8/50).*H. heilmannii* detected in four dogs feaces, one dog fundus, one for each human stool and fundus and one for cat fundus Fig. (2).

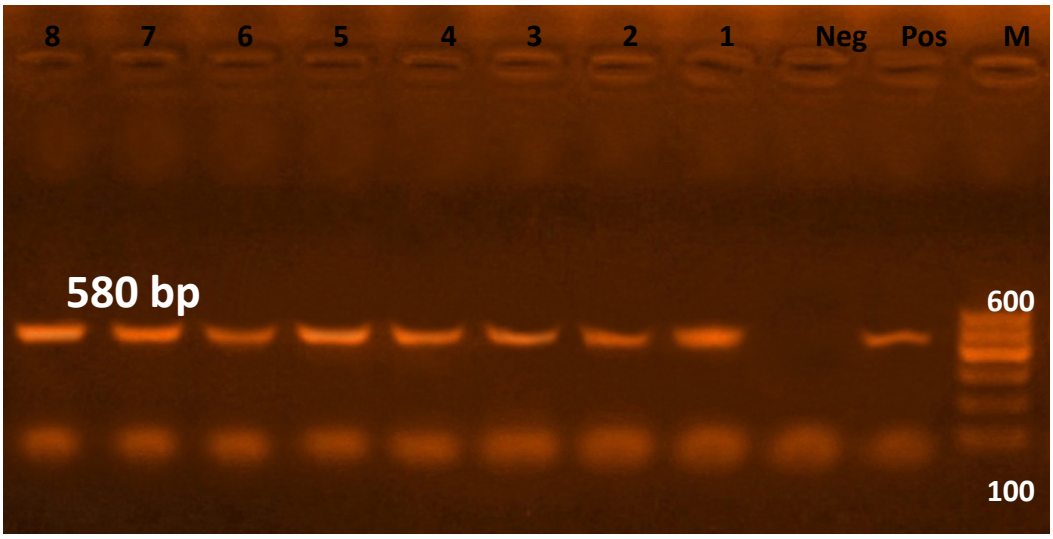

Fig. (2): PCR amplification using *H. heilmannii* ureB primer. The expected size of the product is 580 bp. Lanes: L, 100- 600bp DNA ladder; Pos, DNA extracted from a known positive dog feaces sample; Neg, reagent control (no DNA); 1 - 4, DNA extracted from dog feaces and 5, from dog fundus biopsy; 6, DNA extracted from human stool; 7, DNA extracted from fundus biopsy; 8, DNA extracted from cat fundus biopsy.

Three DNA reveals positive amplified fragments at 296 bp of *H. pylori* based on *H. pylori* glmM primer with prevalence 6% (3/50); two from human fundus and one from dog fundus biopsies.All samples were negative for *H.felis*. Different unidentified *Helicobacter* species are detectable with incidence 78% (39/50) that need further investigation.

DISCUSSION

Helicobacter infection is one of the most common infections worldwide. The incidence of *helicobacter* infection is very high in Egypt mainly *H. pylori* [21]. *Helicobacter* spp. can attack the mucosa of gastrointestinal tract of humans, wild animals such as monkeys and domestic animals (cats, dogs, birds, and pigs) [22,23]. The present investigation detect the different *Helicobacter* spp. in companion animals (dogs and cats) in correlation to zoonotic and public health repertoire. The present research collected 205 samples from apparently healthy companion animals and human patients suffering from gastrointestinal disturbance and in

contact with those animals from Cairo and Giza governorates. The samples were the same collected with pervious authors including faeces, serum and fundus biopsies from dogs and cats as well as human stool and fundus biopsies. [13, 21, 22, 23]. Diagnostic methods for gastric helicobacters were classified as non-invasive or invasive. Noninvasive methods included detection of bacteria, urea breath test (UBT), serologic methods and bacterial DNA. Invasive methods included gastric biopsy specimens or brush cytology, histological examination, electron microscopy, urease test, the polymerase chain reaction techniques (PCR), and in situ hybridization (ISH)[24]. *Helicobacter* spp. is microaerophilic, Gram-negative bacterium, a helix-shaped, able to resist in a highly acid environment due to urease production.

The present work revealed a culture prevalence of 115 different samples were 17.4% with biochemical profile catalase positive, oxidase positive, urea hydrolysis positive, negative nitrate reduction and tolerate 1.25% NaCl that confirmed the non culturable results of some *Helicobacter* spp.[7]. Serological detection of *H. pylori* Antigen or antibodies were common in human lab; seroconversion does not related to degree of inflammation or colonization density [6]. Serologic tests for helicobacteriosis in veterinary are not yet clinically available. Detection of fecal *H. pylori* antigen is possible, but antigen assays have not yet been adapted for use in veterinary patients. The present study reveals high detection of both *H. pylori* Antigen or antibodies in dog feaces then human stool and later cat feaces. The sensitivity and specificity of stool antigen testing against *H. pylori*, is typically exceed 92%, while for *H. pylori* IgG serologic testing has a specificity of less than 80% and cross reaction may occurs [8]. Pathogenesis and therapy questions about *Helicobacter* species require a simple, sensitive, noninvasive, and readily available specific diagnostic test. A polymerase chain reaction (PCR) assay would allow *Helicobacter* documentation in dogs, cats feces aond human stool as well as gastric biopsies from humans and animals [6]. In this study, the authors investigate *Helicobacter* species in dogs, cat's feaces and human stool using PCR against 16S rRNA, *H.heilmannii* ureB, *H. felis* urea, and ureB and *H. pylori* glmM primers with amplified fragments 398 bp, 580 bp, 241 bp and 296 bp respectively. The incidence of *Helicobacter* species were 43.4%. Moreover, *H. heilmannii* was 16% and *H. pylori* were 6%. Detection of *H. heilmannii* in human samples and presence of *H. pylori* in companion animals (dogs and cats) suggested that those animals act as reservoir of *Helicobacter* species; some studies have suggested animals as natural hosts.

This refers to the presence of *H. pylori* in the gastric mucosa of different animal species with mild or absence of an inflammatory response [24].

Also these results support the importance of dogs and cats in transmission of *Helicobacter* species to human especially the patients were in contact with the companion animal under investigation. Many authors documented this theory of zoonotic and public health hazard but not explained the mode of transmission, only suggested by oro-oral or oro-fecal routes.[6, 8]

All samples were *H. felis* negative and 78% of *Helicobacter* species were unidentified that need further investigation. Prevalence studies have suggested that gastric *Helicobacter* infections are almost universally present in both apparently healthy dogs and dogs presenting with clinical gastric disease [6.]

CONCLUSION

From previous data, the study confirmed that serological technique is screening test and PCR is a reliable, sensitive and diagnostic test for detection of *Helicobacter* species. on animals including dogs and cats play a major source as zoonotic and public health hazard. Dogs and cats acts as reservoir with mild or no clinical signs.

REFERENCES

Rappin J. (1958): Contribution á l'étude des bactéries de la bouche á létat normal et dans la fièvre typhoide. Ph.D. Thesis, Collège de France, Nantes, 1881. Ref. Am. J. Vet. Res.; 19: 677-680.

Bizzozero G. (1893): Sulleghiandoletubulari del tubogastroenterico e Sui rapporti Del lorocol Pepiteliodi rivestimentodella mucosa. *Arch MikrAnat*; 42: 82.

Salomon H. (1896): Ueber das Spirillum des Säugetiermagens und sein Verhalten zu den Belegzellen. Zentralbl. Bakteriol. Parasitenkd. Infectionskrankh. 1. Abt.; 19: 433-442.

Lockard V.G. and Boler R.K. (1970): Ultrastructure of a spiraled microorganism in the gastric mucosa of dogs. Am. J. Vet. Res.; 31: 1453-1461.

Owen R.J. *Helicobacter*-species classification and identification. Br. Med. Bull. (1998): 54: 17-30.

Shinozaki J.K., Sellon R.K., Cantor G.H., Besser T.E., Mealey K.L., and Vaden S.L. (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.

Okubo B.M.,Ricci-Azevedo R.,Zobiole N.N.,Buccini D.F. and Moreno S.E.(2017): Prevalence of Helicobacter spp. in dogs from Campo Grande-MS. *Cienc. Anim. bras, Goiânia*, V.18, 1-10.

Crow S.E. (2019): *Helicobacter pylori* Infection. *N Engl J Med* 380; 12: 1158-1165.

Morgner, A.; lehn, N.; Andersen, L. P.; Thiede, C.; Bennedsen, M.; Trebesius, K.; Neubauer, B.; Neubauer, A.; Stlte, M.; Bayerdörfer, E. (2000): *Helicobacter heilmannii*- associated primary gastric low-grade MALT lymphoma: complete remission after curing the infection. *Gastroenterology*, 118:821-828.

Ladeira, M. S. P.; Salvadori, D. M. F.; Rodrigues A. M. and Biopatologia do (2003): *Helicobacter pylori*. *Jornal Brasileiro de Patologia e Medicina Laboratorial*, 39 (4):335-342.

Handt L.K., Fox J.G., Dewhirst F.E., Fraser G.J., Paster B.J., Yan L.L., Rozmiarek H., and Rufo R., Stalis I.H. (1994): *Helicobacter pylori* isolated from the domestic cat: public health implications. *Infect. Immun.*; 62: 2367-2374.

Bulck, K. V. D.; Decostere, A.; Baele, M.; Driessen, A.; Debongnie, J-C.; Burette, A.; Stolte, M.; Ducatelle, R.; Haesebrouck, F. (2005): Identification of Non-*Helicobacter pylori* Spiral Organisms in Gastric Samples from Humans, Dogs, and Cats. Journal of Clinical Microbiology, 43(5):2256–2260.

Hu Y, Wan JH, Li XY, Zhu Y, Graham DY, Lu NH. (2017): Systematic review with Metaanalysis: the global recurrence rate of *Helicobacter pylori*. *Aliment Pharmacol Ther*; 46: 773-9.

Cattoli G, van Vugt R, and Zanoni RG, *et al.* (1999): Occurrence and characterization of gastric *Helicobacter* spp. in naturally infected dogs. *Vet Microbiol*; 70:239-250.

Ford AC, Moayyedi P.(2014) Whom should we "test and treat" for *Helicobacter pylori* BMJ; 348: 3320.

Forbes, B. A., Sahm, D. F. and Weissfeld, A. S. (2007): *Bailey* and *Scott's Diagnostic Microbiology*. (Mosby-Elsevier 2007).

Camargo, P.L.; Alfieri, A.A.; Bracarense, APFRL.; Menoli, R.; Spinosa S.R. and Hagiwara M.K. (2003): Use of Polymerase Chain Reaction and Enzymatic Cleavage in the Identification of *Helicobacter* spp. in Gastric Mucosa of Human Beings from North Paraná, Brazil.

Arfaee F., Jamshidi S., Azimirad M., Dabiri H., Tabrizi A.S. and Zali M.R. (2014): PCR-based diagnosis of Helicobacter species in the gastric and oral samples of stray dogs. *Comp Clin Pathol*, 23:135-139.

Tabrizi A.S., Derakhshandeh A., Esfandiari A. and Atashi Z.A. (2015): Identification of *Helicobacter* spp. in gastrointestinal tract, pancreas and hepatobiliary system of stray cats. *Iran J Vet Res*; 16 (4): 374 - 376.

Sambrook J., Fritscgh E.F and Mentiates (1989): Molecular coloning. A laboratory manual.Vol, Cold spring Harbor Laboratotry press, New York.

Hooi, J. K. Y. *et al.* (2017): Global Prevalence of *Helicobacter pylori* infection**:** systematic review and meta-analysis. *Gastroent.* 153, 420 - 429.

Abdi, F.S.; 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.

Hong, S.; Chung, Y.; Kang, W.G.; Choi, Y.S.; 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. (2016): Detection of gastric *Helicobacter* spp. in stool samples of dogs with *gastritis. Polish Journal of Veterinary Sciences.*19 (2):237–243.

Hatakeyama, M. (2014): .*Helicobacter pylori* cagA and gastric cancer: a paradigm for hit-and-run carcinogenesis. *Cell Host Microbe* 15(3), 306-16.