Genotyping pattern of the vacuolating cytotoxin A and cytotoxin associated gene A of the *Helicobacter pylori* strains detected in fecal samples of household dogs

Asieh Bolandi,¹ Saam Torkan,²
Iman Alavi³

¹Faculty of Veterinary Medicine, University of Tehran; ²Department of Small Animal Internal Medicine, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord; ³Department of Microbiology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Abstract

In despite of the high clinical impact of *Helicobacter pylori*, its exact sources and routes of transmission are unknown. Dogs may play an imperative role in the transmission of *H. pylori* to humans. The current investigation was done to study the status of *vacA* and *cagA* genotypes in the *H. pylori* strains of dogs. One-hundred and fifty fecal samples were collected from healthy and complicated household dogs. Genomic DNA was extracted from fecal samples and presence of *16S rRNA* gene was studied using the PCR amplification. Distribution of *vacA* and *cagA* genotypes were studied by the multiplex PCR. Thirteen out of 150 fecal samples (8.66%) were positive for *H. pylori 16S rRNA* gene. Prevalence of *H. pylori* in healthy and complicated dogs were 5.55% and 8.57%, respectively. Male had the higher prevalence of *H. pylori* (P=0.038). The most commonly detected genotypes among the *H. pylori* strains were *vacAs1A* (61.53%), *cagA* (38.46%), *vacAm1a* (38.46%), *vacAs2* (30.76%) and *vacAm2* (30.76%). The most commonly detected combined genotypes were *s1aCagA* (30.76%), *s1aM1a* (23.07%), *s2M1a* (23.07%) and *s2CagA* (23.07%). Iranian household dogs harbor *H. pylori* in their fecal samples similar in genotypes of the *vacA* and *cagA* alleles which suggest that complicated and even healthy dogs may be the latent host of the *H. pylori* and its genotypes. However, supplementary studies are required to found the exact role of dogs as a definitive host of the *H. pylori*.

Introduction

Dogs are one of the most useful and popular companion animal. Dogs chiefly can affluence loneliness, decrease allergies, depression, anxiety, stress, and variety of human immunological diseases and indorse social interaction, encourage exercise and playfulness, and deliver unqualified love. Dogs are also skilled guard, decent hunters, real narcotic detector and sharp probes. Company of dogs with children can increase their immune levels against many kinds of zoonotic diseases.¹

Helicobacter pylori (*H. pylori*) is a gram negative, spiral shaped bacterium of the human stomach. It is considered as one of the most contentious bacteria in the world. *H. pylori* is responsible for peptic ulcer, gastritis, lymphoma, duodenal ulcer, and gastric cancer.^{2,3} Recorded findings revealed that dogs may play an imperative impact in transmission of *H. pylori* to human.⁴⁻⁶ Gastrointestinal *H. pylori* infections are routine in dogs.⁴⁻⁷ Documented data revealed a high homology in the DNA sequence of *H. pylori* strains isolated from household dogs and also those which cause severe gastroenteritis and other types of gastrointestinal diseases in their owners.⁸ Therefore, close contact with healthy and complicated dogs which harbor *H. pylori* in their stomach has been determined to be a risk factor for human *Helicobacter* infection.⁴⁻⁸

Study the distribution of putative genotypes is essential to assess the microbiological and epidemiological prospective of *H. pylori* in clinical cases. The most imperative virulence genes accompanied with severe cases of *H. pylori* infections are vacuolating cytotoxin A (*vacA*) and cytotoxin associated gene A (*cagA*).^{9,10} The *vacA* belongs to the group of genes with mutable structures. This gene is polymorphic, comprising mutable signal regions (type *s1* or *s2*) and mid-regions (type *m1* or *m2*). The *s1* type is supplementary subtyped into *s1a*, *s1b* and *s1c* and the *m1* into *m1a* and *m1b* subtypes. The mosaic combination of s and m-regions regulates the specific cytotoxin and therefore, the pathogenicity of the *H. pylori*.^{9,10} The *cagA* gene has been detected in the specimens taken from the severe cases of peptic ulcer and gastritis.⁹⁻¹¹ Genotyping using these virulence markers is measured as one of the best methods for assess associations between *H. pylori* isolates from diverse clinical samples.

From clinical, epidemiological, microbiological and hygienic prospective, it is important to assess the distribution of putative genotypes of the *H. pylori* in companion animals like dogs. Considering the

Correspondence: Asieh Bolandi, Faculty of Veterinary Medicine, University of Tehran, Qareeb Street, Azadi Av., P.O. Box 14155-6453, Tehran, Iran.
Tel.: +98.912.0310919.
E-mail: drhodabolandi@gmail.com

Key words: *Helicobacter pylori*, Genotypes, *VacA*, *CagA*, Household dogs.

Acknowledgements: The authors would like to thanks from all of the Small Animal Clinics of the Tehran province for their clinical supports. This work was supported by the authors.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 29 June 2017.

Accepted for publication: 8 August 2017.

This work is licensed under a Creative Commons Attribution NonCommercial 4.0 License (CC BY-NC 4.0).

©Copyright A. Bolandi et al., 2017
Licensee PAGEPress, Italy
Microbiology Research 2017; 8:7289
doi:10.4081/mr.2017.7289

unclear epidemiological aspects of *H. pylori* in fecal samples of dogs, the present investigation was done in order to study the precise status of *vacA* and *cagA* genotypes of *H. pylori* strains detected in the fecal samples of healthy and complicated household dogs.

Materials and Methods

Ethical issues

All samples were taken from volunteer dogs and written signed consent was obtained from the owners of examined dogs. Sampling procedure was non-invasive and all stages of sampling were done in a way that the lowest levels of pain and damages faced to the examined dogs.

Samples

From March 2015 to May 2016, overall 150 fecal samples were collected from the household dogs of different sexes (male and female, age 8 months to 12 years) who were referred for routine checks to the clinics of the Small Animal Internal Medicine, Tehran, Iran. Samples were collected randomly from the healthy dogs (n=90) and those that had history of gastric disorders (n=60). The dogs of the treatment group had a history of gastric ulcer, gastritis, chronic

vomiting and weight loss. Samples were collected in a sterile manner, with care taken to avoid cross-contamination between different sites. Fecal samples were transported to the laboratory at 4°C. All samples were kept under refrigeration in plastic bags.

DNA extraction and *Helicobacter pylori* 16S rRNA gene amplification

Detection of *H. pylori* strains was done based on the 16S rRNA-based PCR amplification. Genomic DNA was extracted from the fecal samples using a DNA extraction kit for cells and tissues (Fermentas, Germany) according to the manufacturer's instructions and its density was assessed by optic densitometry.

Extracted DNA was amplified for the 16S rRNA gene (F: 5'-CTGGAGAGAC-TAAGCCCTCC-3' and R: 5'-ATTACT-GACGCTGATTGTGC-3') (110 bp).¹² PCR reactions were performed in a final volume of 50 µL containing 5 µL 10 × buffer + MgCl₂, 2 mM dNTP, 2 unit Taq DNA polymerase, 100 ng genomic DNA as a template, and 25 picomole of each primer. PCR was performed using a thermal cycler (Eppendorf Co., Germany) under the following conditions: an initial denaturation for 2 minutes at 94°C; 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s and a final extension at 72°C for 8 min.

Genotyping of *vacA* and *cagA* genes of *Helicobacter pylori*

Presence of the *cagA* and the genotypes of *vacA* (*s1a*, *s1b*, *s1c*, *m1a*, *m1b* and *m2*) alleles were determined using PCR technique. List of primers is shown in Table 1.¹³ The PCR was performed in a total volume of 50 µL containing 1 µM of each primer, 1 µL of genomic DNA (approximately 200 ng), 1 mM of dNTPs mix (invitrogen), 2 mM of MgCl₂, and 0.05 U/µL Taq DNA polymerase (invitrogen). PCR amplifications were performed in an automated thermal cycler (Biometra Co., Germany). The following cycle conditions were used for PCR amplification: for *vacA*: 32 cycles of 45s at 95°C, 50s at 64°C, and 70s at 72°C; for *cagA*: 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C. All runs included one negative DNA control consisting of PCR grade water and two or more positive controls (26695, J99, SS1, Tx30, 88-23 and 84-183).

Gel electrophoresis

The PCR amplification products (10 µL) were subjected to electrophoresis in a 1.5% agarose gel in 1X TBE buffer at 80 V for 30 min, stained with ethidium bromide, and images were obtained in a UVIdoc gel

documentation systems (UK). The PCR products were identified by 100 bp DNA size marker (Fermentas, Germany).

Statistical analysis

Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), Chi-square test and Fisher's exact test analysis was performed and differences were considered significant at values of P<0.05. Distribution of *H. pylori* genotypes detected in fecal samples of dogs were statistically analyzed.

Results

Table 2 represents the prevalence of *H. pylori* in of fecal samples of healthy and complicated dogs. Results showed that 13 out of 150 fecal samples (8.66%) were positive for *H. pylori* strains. All of the *H. pylori* strains harbored the 16S rRNA gene. Figure 1 represents the results of the gel

Figure 1. Results of the gel electrophoresis for the 16S rRNA gene of the *H. pylori* strains detected in fecal samples of healthy and complicated dogs. M: 100 bp ladder, 1: Positive control, 2-4: Positive sample for the 16S rRNA gene (110 bp), and 5: Negative control.

Table 1. List of primers used for genotyping of *Helicobacter pylori* strains detected in fecal samples of dogs.

Genes name	Primer Sequence (5'-3')	Size of product (bp)
<i>VacA s_{1a}</i>	F: CTCTCGCTTTAGTAGGAGC R: CTGCTTGAATGCGCCAAAC	213
<i>VacA s_{1b}</i>	F: AGCGCCATACCGCAAGAG R: CTGCTTGAATGCGCCAAAC	187
<i>VacA s_{1c}</i>	F: CTCTCGCTTTAGTGGGGYT R: CTGCTTGAATGCGCCAAAC	213
<i>VacA s₂</i>	F: GCTAACACGCCAAATGATCC R: CTGCTTGAATGCGCCAAAC	199
<i>VacA m1a</i>	F: GGTCAAAATGCGGTCATGG R: CCAITGGTACCTGTAGAAAC	290
<i>VacA m1b</i>	F: GGCCCCAATGCAGTCATGGA R: GCTGTTAGTGCCCTAAAGAAGCAT	291
<i>VacA m₂</i>	F: GGAGCCCCAGGAAACATTG R: CATAACTAGCGCCTTGCA	352
<i>Cag A</i>	F: GATAACAGCCAAAGCTTTTGAGG R: CTGCAAAAGATTGTTTGGCAGA	300

Table 2. Prevalence of *Helicobacter pylori* in different types of fecal samples taken from dogs.

Types of fecal samples	No. samples collected	Prevalence of <i>H. pylori</i> by 16S rRNA gene amplification (%)
Healthy dogs		
Male	40	3 (7.50)
Female	50	2 (4)
Total	90	5 (5.55)
Complicated dogs		
Male	25	5 (20)
Female	35	3 (8.57)
Total	60	8 (13.33)
Total	150	13 (8.66)

Table 3. Distribution of *vacA* and *cagA* genotypes among the *Helicobacter pylori* strains etected in fecal samples of dogs.

Types of samples (N. positive)	Distribution of various genotypes (%)							
	<i>VacA s1a</i>	<i>VacA s1b</i>	<i>VacA s1c</i>	<i>VacA s2</i>	<i>VacA m1a</i>	<i>VacA m1b</i>	<i>VacA m2</i>	<i>CagA</i>
Healthy dogs (5)	2 (40)	1 (20)	-	1 (20)	1 (20)	-	1 (20)	1 (20)
Complicated dogs (8)	6 (75)	2 (25)	1 (12.50)	3 (37.50)	4 (50)	1 (12.50)	3 (37.50)	4 (50)
Total (13)	8 (61.53)	3 (23.07)	1 (7.69)	4 (30.76)	5 (38.46)	1 (7.69)	4 (30.76)	5 (38.46)

electrophoresis for amplification of the *16S rRNA* gene of *H. pylori* strains. Prevalence of *H. pylori* in healthy and complicated dogs were 5.55% and 8.57%, respectively. Statistically significant differences were seen for the prevalence of *H. pylori* between healthy and complicated dogs ($P=0.024$) and also between male and female dogs ($P=0.038$).

Table 3 represents the distribution of *vacA* and *cagA* genotypes among the *H. pylori* strains detected in fecal samples of dogs. *VacAs1A* (61.53%), *cagA* (38.46%), *vacAm1a* (38.46%), *vacAs2* (30.76%) and *vacAm2* (30.76%) were the most commonly detected genotypes among the *H. pylori* strains of dogs. *H. pylori* strains of complicated dogs had the higher prevalence and also more diverse studied genotypes. Statistically significant difference was seen for the prevalence of *H. pylori* genotypes between healthy and complicated dogs ($P=0.035$).

Table 4 represents the distribution of combined genotypes in the *H. pylori* strains of fecal samples of dogs. We found that *s1aCagA* (30.76%), *s1am1a* (23.07%), *s2m1a* (23.07%) and *s2CagA* (23.07%) were the most commonly detected combined genotypes in the *H. pylori* strains detected in fecal samples of dogs. Prevalence of *cagA* positive and *cagA* negative strains were 46.15% and 53.84%, respectively.

Discussion

Humans are recognized as the major host of *H. pylori*; though, the histopathological inspection represents high prevalence and occurrence of *H. pylori* infections in the gastric mucosa of other mammalian which shows their high capacity as latent hosts for *H. pylori* strains.¹⁴

The current research exposed that the dogs and particularly those who had gastric complications like history of gastric ulcer, gastritis, chronic vomiting and weight loss may be the reservoir or original host of the *H. pylori*. Prevalence of *H. pylori* in fecal samples of dogs was 8.66% which was considerable high. Low levels of hygiene used for maintenance of dogs, their close contact

with stray animals and especially dogs and transmission of pathogenic *H. pylori* strains from infected owners to dogs are the main factors caused high prevalence of *H. pylori* in fecal samples of our study. However, the prevalence rate of *H. pylori* could be more than the reported prevalence and it is due the fact that the fecal samples of our study were taken from the dogs who were kept in the houses. In the other hand, all of the dogs of our study had owners and kept in hygienic conditions. In addition, household dogs of our study were fed with cooked foods which were free from *H. pylori* strains. This matters will explain the low prevalence rate of *H. pylori* in fecal samples of dogs in this study. High prevalence of *H. pylori* in raw foods have been reported previously.^{15, 16} Similar findings have been reported previously from Thailand,¹⁷ Iran,⁶ Denmark,¹⁸ Egypt,⁵ Italy⁴ and Belgium.¹⁹

Prevalence rate of *H. pylori* in fecal samples of our investigation (8.66%) was lower than that of Thailand (17.30%).²⁰ Recent researches reported the high prevalence of *Helicobacter*-like microorganism in clinical samples taken from canine. They showed that *Helicobacter*-like organisms are extremely prevalent in dogs, with 100% of random-source dogs,²¹ 67-100% of clinically healthy dogs²¹⁻²⁵ and 100% of laboratory beagles and shelter dogs infected.^{25,26} *Helicobacter* spp. have also been established in gastric biopsies of 61-95% of dogs.^{26,27} Torkan and Sakhaei Shahreza (2016)²⁸ revealed that 13 out of 240 (5.41%) gastric biopsy samples of Iranian dogs were positive for *H. pylori* with a higher prevalence of the bacterium in dogs with gastric ulcer (6.36%). As far as we know, the present study is the first prevalence report of the detection of *H. pylori* in the fecal samples of dogs.

High prevalence of *H. pylori* strains was accompanied with high prevalence of putative genotypes. As it showed, the most commonly detected genotypes were *vacAs1a*, *cagA*, *vacAm1a*, *vacAs2* and *vacAm2*. The only study which was done in this area,²⁸ reported that the prevalence of *vacAs1*, *s1b*, *s1c*, *s2*, *m1a*, *m1b*, *m2* and *cagA* genotypes amongst the *H. pylori* isolates of gastric biopsy samples of Iranian

Table 4. Distribution of combined genotypes amongst the *H. pylori* strains of fecal samples of dogs.

Genotypes	Prevalence (%)*
<i>S1am1a</i>	3 (23.07)
<i>S1Am1b</i>	-
<i>S1am2</i>	2 (15.38)
<i>S1aCagA</i>	4 (30.76)
<i>S1bm1a</i>	1 (7.69)
<i>S1bm1b</i>	-
<i>S1bm2</i>	1 (7.69)
<i>S1bCagA</i>	1 (7.69)
<i>S1cm1a</i>	-
<i>S1cm1b</i>	-
<i>S1cm2</i>	-
<i>S1cCagA</i>	-
<i>S2m1a</i>	3 (23.07)
<i>S2m1b</i>	-
<i>S2m2</i>	2 (15.38)
<i>S2CagA</i>	3 (23.07)
<i>CagA+</i>	5 (46.15)
<i>CagA-</i>	8 (53.84)

*From a total of 13 positive strains of *H. pylori*.

dogs were 53.84%, 23.07%, 0%, 23.07%, 30.76%, 10.81%, 38.46% and 46.15%, respectively which was similar to our results. Torkan and Sakhaei Shahreza (2016)²⁸ in their study revealed that the prevalence of *m1as1a*, *m1as1b*, *m1bs1b*, *m2s1a*, *m2s1b*, *m2s2*, *m1as2*, *m1am2*, *cagA+* and *cgaA-* alleles were 23.07%, 15.38%, 7.69%, 30.76%, 15.38%, 15.38%, 7.69%, 30.76%, 46.15% and 53.84%, respectively which was relatively similar to our percent. High prevalence of the *s1am1a*, *s1am2*, *s2m2* and *s2m1a* genotypes of *H. pylori* have been reported previously in the clinical samples of human beings.²⁹⁻³¹ In addition, *cagA* positive strains have been considered to have a higher pathogenicity for gastric epithelial cells than *cagA* negative strains of *H. pylori*.³² Documented data revealed that *vacA* and *cagA* genotypes are responsible for adhesion, colonization and invasion of *H. pylori* cells to the gastric mucosa. Therefore, their detection and also study their mosaic structure are useful to

found new epidemiological aspects of this bacterium in various clinical samples.

Conclusions

In conclusion, we identified a relatively low prevalence of *H. pylori* in the fecal samples of healthy and complicated dogs in Iran as well as high presence of *vacA* and *cagA* genotypes especially in complicated dogs. Our results indicated that Iranian healthy and complicated household dogs harbor *H. pylori* in their fecal samples similar in genotype of the *vacA* and *cagA* alleles which suggest that complicated and even healthy dogs may be the latent host of the *H. pylori* and its genotypes. This matter is an important public health threat regarding the close contact of human with household dogs. However, additional studies are required to found the exact role of dogs as a definitive host of the *H. pylori*.

References

- Day MJ, Breitschwerdt E, Cleaveland S, et al. Surveillance of zoonotic infectious disease transmitted by small companion animals. *Emerg Infect Dis* 2012;18.
- Ghorbani F, Gheisari E, Dehkordi FS. Genotyping of *vacA* alleles of *Helicobacter pylori* strains recovered from some Iranian food items. *Trop J Pharm Res* 2016;15:1631-6.
- Atapoor S, Dehkordi FS, Rahimi E. Detection of *Helicobacter pylori* in various types of vegetables and salads. *Jundishapur J Microbiol* 2014;7:e10013.
- Recordati C, Gualdi V, Tosi S, et al. Detection of *Helicobacter* spp. DNA in the oral cavity of dogs. *Vet Microbiol* 2007;119:346-51.
- Abdel-Raouf M, Abdel-Gleel Y, Enab A. Study on the role of pet animals for *Helicobacter pylori* transmission. *J Am Sci* 2014;10:20-8.
- Soghra Abdi F, Jamshidi S, Moosakhani F, et al. Detection of *Helicobacter* spp. DNA in the colonic biopsies of stray dogs: molecular and histopathological investigations. *Diagn Pathol* 2014;9:50.
- Wiinberg B, Spohr A, Dietz HH, et al. Quantitative analysis of inflammatory and immune responses in dogs with gastritis and their relationship to *Helicobacter* spp. infection. *J Vet Intern Med* 2005;19:4-14.
- Mousavi S, Safarpour Dehkordi F, Rahimi E. Virulence factors and antibiotic resistance of *Helicobacter pylori* isolated from raw milk and unpasteurized dairy products in Iran. *J Venom Anim Toxins Incl Trop Dis* 2014;20:51.
- Sicinschi LA, Correa P, Bravo LE, et al. Non-invasive genotyping of *Helicobacter pylori* *cagA*, *vacA*, and *hopQ* from asymptomatic children. *Helicobacter* 2012;17:96-106.
- Alikhani MY, Arebestani MR, Khorasani MS, et al. Evaluation of *Helicobacter Pylori vacA* and *cagA* genotypes and correlation with clinical outcome in patients with dyspepsia in Hamadan Province, Iran. *Iran Red Crescent Med J* 2014;16:e19173.
- López-Vidal Y, Ponce-de-León S, Castillo-Rojas G, et al. High diversity of *vacA* and *cagA* *Helicobacter pylori* genotypes in patients with and without gastric cancer. *Plos One* 2008;3:e3849.
- Ho SA, Hoyle JA, Lewis FA. Direct polymerase chain reaction test for detection of *Helicobacter pylori* in humans and animals. *J Clin Microbiol* 1991;29:2543-9.
- Yahaghi E, Khamesipour F, Mashayekhi F, et al. *Helicobacter pylori* in vegetables and salads: genotyping and antimicrobial resistance properties. *Biomed Res Int* 2014;2014:757941.
- Solnick JV, Schauer DB. Emergence of diverse *Helicobacter* species in the pathogenesis of gastric and enterohepatic diseases. *Clin Microbiol Rev* 2001;14:59-97.
- Gilani A, Razavilar V, Rokni N, et al. *VacA* and *cagA* genotypes of *Helicobacter pylori* isolated from raw meat in Isfahan province, Iran. *Vet Res Forum* 2017;8:75-80.
- Atapoor S, Dehkordi FS, Rahimi E. Detection of *Helicobacter pylori* in various types of vegetables and salads. *Jundishapur J Microbiol* 2014;7:e10013.
- Pirarat N, Makbunsri T, Sukkamon S, et al. The relationship between pathological gastric changes and *Helicobacter* spp. in dog. *Thai J Vet Med* 2003;33:73-80.
- Wiinberg B, Spohr A, Dietz HH, et al. Quantitative analysis of inflammatory and immune responses in dogs with gastritis and their relationship to *Helicobacter* spp. infection. *J Vet Intern Med* 2005;19:4-14.
- Van den Bulck K, Decostere A, Baele M, et al. Identification of non-*Helicobacter pylori* spiral organisms in gastric samples from humans, dogs, and cats. *J Clin Microbiol* 2005;43:2256-60.
- Prachasilpchai W, Nuanualsuwan S, Chatsuwan T, et al. Diagnosis of *Helicobacter* spp. infection in canine stomach. *J Vet Sci* 2007;8:139-45.
- Strauss-Ayali D, Simpson KW, Schein AH, et al. Serologic discrimination of dogs infected with gastric *Helicobacter* spp. and uninfected dogs. *J Clin Microbiol* 1999;37:1280-7.
- Neiger R, Tschudi M, Burnens AP, et al. Diagnosis and identification of gastric *Helicobacter* species by PCR in dogs. *Microb Ecol Health Dis* 1999;11:234-40.
- Jalava K, On SL, Vandamme PA, et al. Isolation and identification of *Helicobacter* spp. from canine and feline gastric mucosa. *Appl Environ Microbiol* 1998;64:3998-4006.
- Happonen I, Linden J, Saari S, et al. Detection and effects of helicobacters in healthy dogs and dogs with signs of gastritis. *J Am Vet Med Assoc* 1998;213:1767-74.
- Eaton KA, Dewhirst FE, Paster BJ, et al. Prevalence and varieties of *Helicobacter* species in dogs from random sources and pet dogs: Animal and public health implications. *J Clin Microbiol* 1996;34:3165-70.
- Strauss-Ayali D, Simpson KW, Schein AH, et al. Serologic discrimination of dogs infected with gastric *Helicobacter* spp. and uninfected dogs. *J Clin Microbiol* 1999;37:1280-7.
- Happonen I, Linden J, Saari S, et al. Detection and effects of helicobacters in healthy dogs and dogs with signs of gastritis. *J Am Vet Med Assoc* 1998;213:1767-74.
- Torkan S, Skhvaei Shahreza MH. *VacA*, *CagA*, *IceA* and *OipA* Genotype status of *Helicobacter pylori* isolated from biopsy samples from Iranian dogs. *Trop J Pharm Res* 2016;15:377-84.
- Hosseini E, Poursina F, de Wiele TV, et al. *Helicobacter pylori* in Iran: A systematic review on the association of genotypes and gastroduodenal diseases. *J Res Med Sci* 2012;17:280-92.
- Alikhani MY, Arebestani MR, Sayedin Khorasani M, et al. Evaluation of *Helicobacter pylori vacA* and *cagA* genotypes and correlation with clinical outcome in patients with dyspepsia in hamadan province, Iran. *Iran Red Crescent Med J* 2014;16:e19173.
- Havaei SA, Mohajeri P, Khashei R, et al. Prevalence of *Helicobacter pylori vacA* different genotypes in Isfahan, Iran. *Adv Biomed Res* 2014;3:48.
- Jafari F, Shokrzadeh L, Dabiri H, et al. *vacA* genotypes of *Helicobacter pylori* in relation to *cagA* status and clinical outcomes in Iranian populations. *Jpn J Infect Dis* 2008;61:290-3.