VacA and cagA genotypes of Helicobacter pylori isolated from raw meat in Isfahan province, Iran

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Article Info

Abstract

Foods with animal origins play a substantial role in the transmission of Helicobacter pylori. The present investigation was carried out to study the vacA and cagA genotypes status of H. pylori isolated from various types of meat samples. Two hundred and twenty meat samples were collected and cultured. H. pylori -positive strains were analyzed for the presence of vacA and cagA genotypes. Eleven out of 220 (5.00%) samples were positive for H. pylori. Findings were confirmed by nested PCR. Prevalence of H. pylori in the meat samples of slaughterhouses and butcheries were 72.20% and 27.70%, respectively. The most commonly detected genotypes in the meat samples of slaughterhouses and butcheries were vacA m1a (66.66%) and vacA s1a (37.50%), respectively. The S1a:m1a was the most commonly detected genotype. Meat sampled from butcheries had the higher prevalence of H. pylori and its genotypes than those of slaughterhouses (p < 0.05). Results showed that meat samples could be the potential sources of virulent strains of H. pylori. Application of sanitary measures in the storage, transportation and sale of meat is essential for reducing the levels of H. pylori cross contamination.

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Key words: Butchery, Genotype, Helicobacter pylori, Meat, Slaughterhouse

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Introduction

Meat as the most valuable livestock product is composed of protein and amino acids, minerals, fats and fatty acids, vitamins and other bioactive components which are beneficial for human health. From the nutritional point of view, meat’s importance is derived from its high quality protein, containing all essential amino acids and highly bioavailable minerals and vitamins. In addition, presence of essential fatty acids multiplies the nutritional value of this food.1,2 In keeping with this, meat can convert into the luscious, energetic and graceful products like sausage and hamburger. Due to the high consumption rate of meat and meat products in the world, observation of sanitary conditions is critical in their production and distribution.

*Helicobacter pylori* is a microaerophilic gram negative and spiral shaped bacterium which its main reservoir is humans, predominantly the human stomach. It colonizes most of the population, making it one of the most provocative bacteria in the world. It is the most suspected cause of peptic ulcer disease, duodenal ulcer, type B gastritis, gastric adenocarcinoma and mucosa-associated lymphoid tissue and lymphoma.3 It has been estimated that 17 to 86.00% of hospitalized patients with peptic ulcers were infected with *H. pylori*.3,5

The main routes of *H. pylori* infection have not been identified yet.6 However, it is prospective that *H. pylori* infection occurs throughout childhood or adolescence both in developed and developing countries.5 The main routes for transmission of *H. pylori* are person to person contacts especially fecal-oral or oral-oral.6

The role of foods in the transmission of *H. pylori* is still unknown but there were several novel investigations focused on the identification of this bacterium in various types of food samples.6-10 Suitable conditions like appropriate pH, moisture and temperature cause *H. pylori* to be easily survive in various types of foods including vegetable, salad, milk and meat.8,10

To appraise the pathogenicity of *H. pylori*, evaluation of latent virulence factors and genotypes is essential. The most commonly important virulence factors among *H. pylori* strains of different clinical outcomes of human and animal are the vacuolating cytotoxin (*vacA*) and cytotoxin associated gene (*cag*).8,11,12 These genes usually induce adhesion and invasion to the gastric epithelial cells.13,14 The *vacA* belongs to the group of genes with variable genotypes or structures. This gene is associated with injury to epithelial cells. The *vacA* gene is polymorphic, comprising variable signal regions (type *s1* or *s2*) and mid-regions (type *m1* or *m2*). The *s1* type is further subtyped into *s1a*, *s1b* and *s1c* subtypes and the *m1* into *m1a* and *m1b* subtypes. The mosaic combination of *s* and *m*-region allelic types determines the particular cytotoxin and consequently, the pathogenicity of the bacterium.13,15

The *Cag* pathogenicity island (PAI) has been shown to be involved in persuading inflammation, ulceration and carcinogenesis.14 The *cagA* gene has been detected in the specimens taken from the severe cases of peptic ulcer.10-14 Genotyping using these virulence markers is considered as one of the best approaches for study of correlations between *H. pylori* isolates from different samples.

Data on the epidemiology and transmission of *H. pylori* are extremely significant to prevent its distribution and identify high-risk populations, especially in areas that have high rates of gastritis, peptic ulcers and gastric cancer like Iran.8,12,15 Considering the unclear epidemiological aspects of *H. pylori* in meat and meat products and due to the high prevalence of *H. pylori* all around the world, the present investigation was carried out in order to study the exact status of *vacA* and *cagA* genotypes of *H. pylori* isolated from various types of meat and meat products.

Materials and Methods

Sample collection. From July to September 2015, overall 220 meat samples including beef (n = 80), mutton (n = 70) and chevon (n = 70) were collected from slaughterhouses and butcheries of various parts of Isfahan province, Iran. Samples (100 mg, in sterile glass containers) were transferred to laboratory at 4 °C and then refrigerated in plastic bags; information about dates of production and of assigned shelf lives was not presented.

Isolation of *H. pylori*. Each homogenized sample (25 g) was added to 225 mL of Wilkins Chalgren anaerobe broth (Oxoid Ltd., Basingstoke, UK) supplemented with colistin methanesulfonate (30 mg L⁻¹) and 5% of horse serum (Sigma, St. Louis, USA) and 30 mg L⁻¹ nalidixic acid, 10 mg L⁻¹ vancomycin, 100 mg L⁻¹ cyclheximide and 30 mg L⁻¹ trimethoprim (Sigma) and incubated for 7 days at 37 °C with shaking under microaerophilic conditions (5% oxygen, 85% nitrogen and 10% CO₂) using MART system (Anoxamat, Lichtenwoorde, The Netherland). Then, 0.10 mL of the enrichment selective broth was plated onto Wilkins Chalgren anaerobe agar (Oxoid) supplemented with 5% of defibrinated horse blood and 30 mg L⁻¹ colistin methanesulfonate, 100 mg L⁻¹ cyclheximide 30 mg L⁻¹ nalidixic acid, 30 mg L⁻¹ trimethoprim, and 10 mg L⁻¹ vancomycin (Sigma) and incubated for seven days at 37 °C under microaerophilic conditions (5% oxygen, 85% nitrogen, and 10% CO₂) using MART system (Anoxamat). For comparison, a reference strain of *H. pylori* (ATCC 43504) was employed.

DNA extraction and nested PCR amplification. Typical colonies of *H. pylori* were further identified using nested-PCR method. Genomic DNA was extracted from typical colonies using a DNA extraction kit for cells and tissues (Fermentas, St. Leon-Rot, Germany) according to the manufacturer’s instructions and its density was assessed by optic densitometry. The first and second steps of PCR was performed based on the method described previously.16
Genotyping of vacA and cagA genes of H. pylori. Presence of the vacA and cagA alleles were determined using PCR technique. All PCR reactions were done using the programmable thermal cycler (Eppendorf Co., Hamburg, Germany). 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). A PCR method used for amplification of various genotypes of vacA was done with a total volume of 50 µL including 2 mM MgCl2, 1 µM of forward primer, 1 µM of reverse primer, 5 µL PCR buffer 10X, 200 µM dNTP (Fermentas), 1 U Taq DNA polymerase (Fermentas) and 2.50 µL DNA template. The DNA was then amplified by 32 successive cycles of denaturation at 95 °C for 45 sec, primer annealing at 64 °C for 50 sec and DNA chain extension at 72 °C for 70 sec. Similar PCR reaction was done for the cagA genotype. Amplified DNA of cagA PCR method was then amplified by 32 successive cycles of denaturation at 95 °C for 60 sec, primer annealing at 56 °C for 60 sec and DNA chain extension at 72 °C for 60 sec.

Gel electrophoresis. The PCR amplification products (10 µL) were subjected to electrophoresis in a 2% agarose gel in 1X TBE buffer at 80 V for 30 min, stained with SYBR Green and images were obtained in a UVI doc gel documentation systems (Uvitec Limited, Cambridge, UK). The PCR products were identified by 100 bp DNA size marker (Fermentas).

Statistical analysis. Data were transferred to Microsoft Excel spreadsheet (version 16; Microsoft Corp., Redmond, USA) for analysis. Using statistical software (version 16; SPSS Inc., Chicago, USA), Chi-square test and Fisher's exact two-tailed test analysis were performed and differences were considered significant at values of p < 0.05. Distribution of H. pylori genotypes isolated from meat samples was statistically analyzed.

Results

Table 1 represents the total prevalence of H. pylori in various types of meat samples. Eleven out of 220 (5.00%) meat samples were positive for the H. pylori in the culture-based method. The results of culture method were confirmed by nested PCR technique. Total prevalence of H. pylori in meat of slaughterhouses and butcheries were 2.72% and 7.27%, respectively.

| Type of samples | No. of samples | No. of H. pylori in culture (%) | No. of H. pylori confirmed in nested PCR (%) |
|-----------------|----------------|--------------------------------|--------------------------------------------|
| **Slaughterhouses** |               |                                |                                            |
| Beef            | 40             | -                              | -                                          |
| Mutton          | 35             | 2 (5.71)                       | 2 (5.71)                                  |
| Chevon          | 35             | 1 (2.85)                       | 1 (2.85)                                  |
| Total           | 110            | 3 (2.72)                       | 3 (2.72)                                  |
| **Butcheries**  |               |                                |                                            |
| Beef            | 40             | 2 (5/00)                       | 2 (5/00)                                  |
| Mutton          | 35             | 4 (11.42)                      | 4 (11.42)                                 |
| Chevon          | 35             | 2 (5.71)                       | 2 (5.71)                                  |
| Total           | 110            | 8 (7.27)                       | 8 (7.27)                                  |

Totally, mutton from butcheries (11.42%), mutton from slaughterhouses (57.1%) andchevon from butcheries (5.71%) had the highest prevalence of H. pylori. The meat samples taken from butcheries had the higher prevalence of H. pylori than those of slaughterhouses (p < 0.05).

Table 2 shows the distribution of various genotypes in H. pylori strains of meat and meat products. The most commonly detected genotypes in the meat samples taken from butcheries and those of slaughterhouses were vacA m1a (66.66%) and vacA s1a (37.50%), respectively. The H. pylori strains of butcher’s meat samples harbored the higher prevalence of all studied genotypes than those of slaughterhouses (p < 0.05).

Table 3 reveals the distribution of combined genotypes in the H. pylori strains of meat and meat products. The S1am1a was the most commonly detected genotype in the H. pylori strains of all studied samples. The most commonly detected genotype in the H. pylori strains of slaughter-houses and butcheries were s1am1a, too.

Discussion

The results of the present investigation revealed that the meat samples of slaughterhouses and butcheries were contaminated with virulent strains of H. pylori. We found that the meat samples of butcheries had the higher prevalence of H. pylori. However, the main reason for this finding is uncertain but it seems that cross contaminations of meat samples after slaughter are maybe the main factor for the higher prevalence of H. pylori in butcheries. Increase in the prevalence of human-based genotypes in the meat samples from butcheries is another reason for the possibility of cross contamination. In fact, meat washing, storing and transporting are the main stages which may increase the prevalence of H. pylori contamination. Reports showed that H. pylori can survive in water. In addition, the number of investigations which were focused on the isolation of H. pylori from the water samples are significant. The lack of drinking water for washing of animal carcass and using unsanitary water in slaughterhouses is one of the main factors for presence of H. pylori in meat samples. Transmission of H. pylori strains form the hands of infected staff and also butcheries is another reason for high prevalence of H. pylori. Possibility for contamination of animal carcasses in.
Table 2. Distribution of various genotypes in *Helicobacter pylori* strains of meat samples.

| Types of samples (No. positive samples) | Distribution of genotypes (%) | CagA |
|----------------------------------------|--------------------------------|------|
|                                        | VacA                          |      |
|                                        | S1a  | S1b  | S1c  | S2   | M1a  | M1b  | M2   |      |
| Slaughterhouses                        |      |      |      |      |      |      |      |      |
| Mutton (2)                             | 1 (50.00) | -   | -   | -   | 1 (50.00) | -   | -   |      |
| Chevon (1)                             | -   | -   | -   | -   | 1 (100) | -   | -   |      |
| Total (3)                              | 1 (33.33) | -   | -   | -   | 2 (66.66) | -   | -   |      |
| Butcheries                             |      |      |      |      |      |      |      |      |
| Beef (2)                               | 1 (50.00) | -   | -   | -   | 1 (50.00) | -   | -   |      |
| Mutton (4)                             | 1 (25.00) | -   | -   | 1 (25.00) | -   | 1 (25.00) | 1 (25.00) |      |
| Chevon (2)                             | 1 (50.00) | -   | -   | -   | -   | -   | -   | 1 (50.00) |
| Total (8)                              | 3 (37.50) | -   | 1 (12.50) | 1 (12.50) | 1 (12.50) | 2 (25) |      |

Table 2 shows the impact of meat consumption on the prevalence of *Helicobacter pylori* strains in meat samples. The second part of our investigation was focused on the vacA and cagA genotype status of *H. pylori* isolates of meat samples. Our results showed that cagA, vacA s1a, vacA m1a, vacA s2 and vacA m2 were the most commonly detected genotypes. In addition, s1am1a, s1am2, s2m1a and s2m2 were the most commonly detected combined genotypes. Unfortunately, the number of studies which were focused on the genotyping of *H. pylori* in food stuffs is low. A recent Iranian investigation conducted by Yahaghi et al. showed that from 380 vegetable and 50 salad samples, 52 (13.68%) and 7 (14.00%) were positive for *H. pylori*, respectively. The most commonly detected genotypes in their investigation were oipA (86.44%), cagA (57.62), vacA s1a (37.28%) and vacA m1a (30.50%). Their results showed that vacA s1c had the lowest prevalence which was similar to our findings. Moussavi et al. showed that the most commonly detected genes in the *H. pylori* strains recovered from milk and dairy products were cagA (76.60%) and vacA (75.00%) which was similar to our findings. Hemmatinezhad et al. reported that 13.45% of ready to eat food samples were positive for *H. pylori*. Meat-based ready to eat food samples were the most contaminated. They revealed that the most commonly detected genotypes were vacA s1a (78.37%), vacA m2 (75.67%), vacA m1a (51.35%) and cagA (41.89%) which was similar to our findings. They showed that s1am2 (70.27%), s1am1a (39.18%) and m1am2 (31.08%) were the most commonly detected combined genotypes which was similar to our investigation. In a study which was conducted by Saedi and Sheikhshahrkoh 820 meat and milk samples were studied for presence of *H. pylori* and vacA genotypes. They showed that the prevalence of *H. pylori* in meat samples was 26.25% which was higher than our results. Total prevalence of *H. pylori* in cow, sheep, goat, buffalo and camel meat samples was 25.00%, 37.00%, 22.00%, 28.00% and 14.00%, respectively. s1a and m1a were the most commonly detected genotypes. The most commonly detected combined genotypes were m1as1a (68.52%), m1as1b (60.40%), m1bs1b (55.83%) and m1bs1a (53.29%) which was similar to our findings.

The CagA, vacA s1a, m1a and m2 were the most commonly detected genotypes in the clinic food samples,^8,10,27,28^ those of animal sources^8,21^ and from human beings.^29-31^
Close association of vacA and cagA genotypes with production of interleukin-8 and cytotoxin, gastric epithelial cells adhesion, inflammatory effects, vacuolization and apoptosis in gastric epithelial cells has been observed previously. Since \textit{H. pylori} isolates in our study harbored vacA and cagA genotypes, consumption of meat contaminated with virulent strains may provoke duodenal ulceration, gastric mucosal atrophy and gastric cancer.

In conclusion, Iranian meat samples harbored \textit{H. pylori} strains with high prevalence of vacA and cagA genotypes. High prevalence of \textit{H. pylori} in our samples suggests that contaminated meat samples maybe the sources of bacteria and can transmit to the human population. Diversity of \textit{H. pylori} genotypes between various types of meat samples showed that there might be various sources of contamination. The most important finding of this study is that the meat samples of our research harbored virulent strains of \textit{H. pylori}. A robust health protocol on hygiene of slaughterhouses and the butcheries can reduce the risk of transmission of \textit{H. pylori} from meat and its products to human.

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References

1. Pereira PM, Vicente AF. Meat nutritional composition and nutritive role in the human diet. Meat Sci 2013; 93(3): 586-592.
2. Valsta LM, Tapanainen H, Männistö S. Meat fats in nutrition. Meat Sci 2005; 70(3): 525-530.
3. Shrestha S, Paudel P, Pradhana GB, et al. Prevalence study of \textit{H. pylori} infection in dyspeptic patients coming to Nepal Medical College Teaching Hospital, Jorpati, Kathmandu. Nepal Med Coll J 2012; 14(3): 229-233.
4. Mastromarino P, Conti C, Donato K, et al. Does hospital work constitute a risk factor for \textit{Helicobacter pylori} infection? J Hosp Infect 2005; 60(3): 261-268.
5. Vu C, Ng YY. Prevalence of \textit{Helicobacter pylori} in peptic ulcer disease in a Singapore hospital. Singapore Med J 2000; 41(10): 478-481.
6. Webberley MJ, Webberley JM, Newell DG, et al. Seroepidemiology of \textit{Helicobacter pylori} infection in vegans and meat-eaters. Epidemiol Infect 1992; 108(3): 457-462.
7. Stevenson TH, Bauer N, Lucia LM, et al. Attempts to isolate \textit{Helicobacter} from cattle and survival of \textit{Helicobacter pylori} in beef products. J Food Prot 2000; 63(2): 174-178.
8. Mousavi S, Safarpoor-Dehkordi F, Rahimi E. Virulence factors and antibiotic resistance of \textit{Helicobacter pylori} isolated from raw milk and unpasteurized dairy products in Iran. J Venom Anim Toxins Incl Trop Dis 2014; 20: 51-57.
9. Atapoor S, Safarpoor-Dehkordi F, Rahimi E. Detection of \textit{Helicobacter pylori} in various types of vegetables and salads. Jundishapur J Microbiol 2014; 7(5): doi: 10.5812/jjm.10013
10. Yahaghi E, Khamesipour F, Mashayekhi F, et al. \textit{Helicobacter pylori} in vegetables and salads: genotyping and antimicrobial resistance properties. Biomed Res Int 2014; 2014: 1-11.
11. Montaz H, Dabiri H, Souod N, et al. Study of \textit{Helicobacter pylori} genotype status in cows, sheep, goats and human beings. BMC Gastroenterol 2014; 14: 1-7.
12. Montaz H, Souod N, Dabiri H, et al. Study of \textit{Helicobacter pylori} genotype status in saliva, dental plaques, stool and gastric biopsy samples. World J Gastroenterol 2012; 18(17): 2105-2111.
13. Torres LE, Melián K, Moreno A, et al. Prevalence of vacA, cagA and babA2 genes in Cuban \textit{Helicobacter pylori} isolates. World J Gastroenterol 2009; 15(2): 204-210.
14. Yamazaki S, Yamakawa A, Okuda T, et al. Distinct diversity of vacA, cagA, and cagE genes of \textit{Helicobacter pylori} associated with peptic ulcer in Japan. J Clin Microbiol 2005; 43(8): 3906-3916.
15. Montaz H, Souod N, Dabiri H. Comparison of virulence factors of \textit{Helicobacter pylori} isolated in stomach and saliva in Iran. Am J Med Sci 2010; 340(5): 345-349.
16. Yamada R, Yamaguchi A, Shibasaki K. Detection and analysis of Helicobacter pylori DNA in the gastric juice, saliva, and urine by nested PCR. Oral Sci Int 2008; 5(1): 24-34.
17. Chomvarin C, Namwat W, Chaicumpar K, et al. Prevalence of Helicobacter pylori vacA, cagA, cagE, iceA and babA2 genotypes in Thai dyspeptic patients. Int J Infect Dis 2008; 12(1): 30-36.
18. Ben Mansour K, Fendri C, Zribi M, et al. Prevalence of Helicobacter pylori vacA, cagA, iceA and oipA genotypes in Tunisian patients. Ann Clin Microbiol Antimicrob 2010; 9: 1-7.
19. Ranjbar R, Khamseipour F, Jonaidi-Jafari N, et al. Helicobacter pylori isolated from Iranian drinking water: vacA, cagA, iceA, oipA and babA2 genotype status and antimicrobial resistance properties. FEBS Open Bio 2016; 6(5): 433-441.
20. Ranjbar R, Khamseipour F, Jonaidi-Jafari N, et al. Helicobacter pylori in bottled mineral water: Genotyping and antimicrobial resistance properties. BMC Microbiol 2016; 16: 40.
21. Rahimi E, Kheirabadi EK. Detection of Helicobacter pylori in bovine, buffalo, camel, ovine, and caprine milk in Iran. Foodborne Pathog Dis 2012; 9(5): 453-456.
22. Fujimura S, Kawamura T, Kato S, et al. Detection of Helicobacter pylori in cow’s milk. Lett Appl Microbiol 2002; 35(6): 504-507.
23. Quagli NC, Dambrosio A, Normanno G, et al. High occurrence of Helicobacter pylori in raw goat, sheep and cow milk inferred by glmM gene: A risk of foodborne infection? Int J Food Microbiol 2008; 124(1): 43-47.
24. Angelidis AS, Tirodimos I, Bobos M, et al. Detection of Helicobacter pylori in raw bovine milk by fluorescence in situ hybridization (FISH). Int J Food Microbiol 2011; 151(2): 252-256.
25. Dore MP, Sepulveda AR, El-Zimaity H, et al. Isolation of Helicobacter pylori from sheep—implications for transmission to humans. Am J Gastroenterol 2011; 96(5): 1396-1401.
26. Mhaskar RS, Ricardo I, Azliyati A, et al. Assessment of risk factors of Helicobacter pylori infection and peptic ulcer disease. J Glob Infect Dis 2013; 5(2): 60-67.
27. Hemmatinezhad H, Montaz H, Rahimi E. VacA, cagA, iceA and oipA genotypes status and antimicrobial resistance properties of Helicobacter pylori isolated from various types of ready to eat foods. Ann Clin Microbiol Antimicrob 2016; 15: 1-9.
28. Saeidi E, Sheikhhshahroikh AH. VacA genotype status of Helicobacter pylori isolated from foods with animal origin. Biomed Res Int 2016; 2016: 1-6.
29. 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(11): doi: 10.5812/ircmj.19173.
30. Havaei SA, Mohajeri P, Khashei R, et al. Prevalence of Helicobacter pylori vacA different genotypes in Isfahan, Iran. Adv Biomed Res 2014; 3: 48.
31. Miftahussurur M, Sharma RP, Shrestha PK, et al. Molecular epidemiology of Helicobacter pylori infection in Nepal: Specific Ancestor Root. Plos One 2015; 10(7):doi:10.1371/journal.pone.0134216.
32. Sahara S, Sugimoto M, Vilaichone RK. Role of Helicobacter pylori cagA EPIYA motif and vacA genotypes for the development of gastrointestinal diseases in Southeast Asian countries: a meta-analysis. BMC Infect Dis 2012; 12: 1-13.
33. Karlsson A, Ryberg A, Nosouhi Dehnoei M, et al. Association between cagA and vacA genotypes and pathogenesis in a Helicobacter pylori infected population from South-eastern Sweden. BMC Microbiol 2012; 12: doi:10.1186/1471-2180-12-129.