

Artículo de investigación

Sero-epidemiologic study of helicobacter pylori in cowherds of Shahrekord province

Estudio sero-epidemiológico de helicobacter pylori en pastos de vacas de la provincia de Shahrekord

Estudo sero-epidemiológico de helicobacter pylori em vacas da província de Shahrekord

Recibido: 16 de enero de 2019. Aceptado: 06 de febrero de 2019

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Abstract

Helicobacter pylori (*H. pylori*) is a common infection in developing countries, causing chronic gastritis, peptic ulcer disease, gastric malignancies. Its main transmission methods include human-human transmission, especially in early childhood, environmental (water supply and hygiene) and zoonotic factors. Some researchers have suggested that pigs, cats, and dogs are infected with *Helicobacter* and sheep is suggested as an important animal reservoir of *H. pylori*, causing infection in shepherds. Accordingly, cowherds might also be prone to *H. pylori* infection, while studies have scarcely addressed this issue. Thus, we aimed to investigate the prevalence of *H. pylori* infection in cowherds of Shahrekord province. In this cross-sectional study, conducted from June to October 2016, 80 cowherds with at least three days a week involvement with cows were compared with a matched control group from their neighbors with no direct contact with cows. Demographic information, including age, sex, job experience, educational level, number of family members, history of cancer or peptic ulcer, gastric symptoms, including epigastric pain, nausea/vomiting, and heartburn were collected. One blood sample was taken from all participants for ELISA measurement of *H. pylori*. Mean \pm SD age of participants was 35.8 ± 14.3 ($P > 0.05$). Serum examination of *H. pylori* showed positive

Resumen

Helicobacter pylori (*H. pylori*) es una infección común en los países en vías de desarrollo, que causa gastritis crónica, enfermedad de úlcera péptica y neoplasias gástricas. Sus principales métodos de transmisión incluyen la transmisión humano-humano, especialmente en la primera infancia, los factores ambientales (suministro de agua e higiene) y zoonóticos. Algunos investigadores han sugerido que los cerdos, gatos y perros están infectados con *Helicobacter* y se sugiere la presencia de ovejas como un importante reservorio animal de *H. pylori*, que causa infección en los pastores. En consecuencia, los pastos de ganado también podrían ser propensos a la infección por *H. pylori*, mientras que los estudios apenas han abordado este problema. Por lo tanto, el objetivo fue investigar la prevalencia de la infección por *H. pylori* en los pastos de vacas de la provincia de Shahrekord. En este estudio transversal, realizado de junio a octubre de 2016, se compararon 80 rebaños de ganado con al menos tres días a la semana de participación con vacas con un grupo de control pareado de sus vecinos sin contacto directo con las vacas. Se recopiló información demográfica, incluyendo edad, sexo, experiencia laboral, nivel educativo, número de miembros de la familia, antecedentes de cáncer o úlcera péptica, síntomas gástricos, incluido dolor epigástrico, náuseas / vómitos y acidez estomacal. Se tomó

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IgG in 89 participants (55.6%), which was 57.5% in the case group and 53.8% in the control group ($P > 0.05$). The prevalence of gastric symptoms was not statistically significant different between the groups and there was also no association between presence of *H. pylori* and gastric symptoms or demographic variables, including age, sex, job, educational level, marital status, and number of family members ($P > 0.05$). Cowherds have the same prevalence of *H. pylori* infection than the control group that suggests that cows are not a potential zoonotic factor for *H. pylori* infection in cowherds.

Keywords: Helicobacter pylori, Iran, Cattle, Sero-epidemiologic, peptic ulcer,

una muestra de sangre de todos los participantes para la medición ELISA de *H. pylori*. La edad media \pm desviación estándar de los participantes fue de 35.8 ± 14.3 ($P > 0.05$). El examen sérico de *H. pylori* mostró IgG positiva en 89 participantes (55,6%), que fue del 57,5% en el grupo de casos y del 53,8% en el grupo control ($P > 0,05$). La prevalencia de síntomas gástricos no fue estadísticamente significativa diferente entre los grupos y tampoco hubo asociación entre la presencia de *H. pylori* y los síntomas gástricos o variables demográficas, incluyendo edad, sexo, empleo, nivel educativo, estado civil y número de miembros de la familia. ($P > 0.05$). Los pastos de vacas tienen la misma prevalencia de infección por *H. pylori* que el grupo de control que sugiere que las vacas no son un factor zoonótico potencial para la infección por *H. pylori* en los pastos de vacuno.

Palabras claves: Helicobacter pylori, Irán, Bovinos, Sero-epidemiológicos, úlcera péptica.

Resumo

Helicobacter pylori (*H. pylori*) é uma infecção comum em países em desenvolvimento, causando gastrite crônica, úlcera péptica, neoplasias gástricas. Seus principais métodos de transmissão incluem a transmissão humano-humana, especialmente na infância, fatores ambientais (abastecimento de água e higiene) e zoonóticos. Alguns pesquisadores sugeriram que porcos, gatos e cães são infectados com *Helicobacter* e que ovinos são sugeridos como um importante reservatório animal de *H. pylori*, causando infecção em pastores. Conseqüentemente, os pastores de vacas também podem ser propensos à infecção por *H. pylori*, enquanto os estudos mal abordaram essa questão. Assim, nosso objetivo foi investigar a prevalência da infecção por *H. pylori* em vacas da província de Shahrekord. Neste estudo transversal, conduzido de junho a outubro de 2016, 80 vacas com pelo menos três dias por semana de envolvimento com vacas foram comparados com um grupo controle pareado de seus vizinhos sem contato direto com vacas. Informações demográficas, incluindo idade, sexo, experiência de trabalho, nível educacional, número de membros da família, história de câncer ou úlcera péptica, sintomas gástricos, incluindo dor epigástrica, náusea / vômito e azia foram coletados. Uma amostra de sangue foi coletada de todos os participantes para a medição de ELISA do *H. pylori*. A idade média \pm DP dos participantes foi de $35,8 \pm 14,3$ anos ($P > 0,05$). O exame sérico de *H. pylori* mostrou IgG positiva em 89 participantes (55,6%), que foi de 57,5% no grupo caso e 53,8% no grupo controle ($P > 0,05$). A prevalência de sintomas gástricos não foi estatisticamente significativa entre os grupos e também não houve associação entre a presença de *H. pylori* e sintomas gástricos ou variáveis demográficas, incluindo idade, sexo, emprego, nível educacional, estado civil e número de membros da família. ($P > 0,05$). Cowherds têm a mesma prevalência de infecção por *H. pylori* do que o grupo controle, o que sugere que as vacas não são um potencial fator zoonótico para infecção por *H. pylori* em vacas.

Palavras-chave: Helicobacter pylori, Irã, Gado, Seroepidemiológica, úlcera péptica.

Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that affects a large number of patients worldwide (Perez-Perez et al, 2004). Although its prevalence has decreased in the past decade in developed countries (Tkachenko et al. 2007), it still has a high prevalence in developing

countries and low socio-economic communities, especially in Iran with an overall prevalence estimated of more than 50% (Moosazadeh et al, 2016; Sayehmiri et al, 2014). Some cities have reported a prevalence rate of greater than 80% (Alborzi et al, 2006), associated to high

prevalence of gastric cancer in North and North-West cities of Iran (Malekzadeh et al, 2009; Malekzadeh et al, 2004).

Although *H. pylori* infection is usually asymptomatic and silent, it is the most common cause of chronic gastritis, peptic ulcer disease, gastric malignancies and other diseases that cause abdominal pain, vomiting, anemia, and other complications (Pacífico et al, 2010).

Generally, diagnosis of *H. pylori* infection include invasive and noninvasive methods, comprising serology, immunoblot, stool antigen test, and urea breath test (UBT). Invasive tests include histological examination, polymerase chain reaction (PCR) for antibiotic-resistant types of *H. pylori*, and rapid urease test (RUT) (Monteiro et al, 2001). As determined in an Iranian study, serological examination by enzyme-linked immunosorbent assay (ELISA) is the most accurate diagnostic method available, with a sensitivity of 52.3% (4). After appropriate diagnosis, *H. pylori* infection can be successfully eradicated in most patients, but we need to know more about transmission factors for preventing re-infection (Fakheri et al, 2014).

Despite numerous studies focusing on *H. pylori* infection, its transmission methods are still unclear; the risk factors associated with *H. pylori* infection in the Iranian population include higher age, female sex, larger family size, source of water supply, level of education and hygiene in Iran, while its prevalence was not different between rural and urban areas in Iran (Eshraghian, 2014). As proposed, its main transmission is human-human transmission, especially in early childhood through fecal-oral, gastro-oral, and close-contact routes (Das & Paul, 2007) and some have suggested the intrafamilial transmission (Kivi & Tindberg, 2006). Although, human-human transmission is suggested as the main transmission method, environmental and zoonotic factors are also the proposed as possible transmission pathways (Brown, 2000; Goodman & Correa, 1995); but, few studies have focused on the zoonotic transmission. Some researchers have suggested that pigs, cats and dogs are infected with other types of *Helicobacter* (Neiger & Simpson, 2000; Pozdeev et al, 2014). Other studies have also identified *H. pylori* in cow and sheep milk (Quaglia et al, 2008), feces (Safaei et al, 2011), and gastric specimens (Montaz et al, 2014). Therefore, sheep is suggested an important animal reservoir of *H. pylori*, causing infection in

shepherds (Papież et al, 2003; Dore, 2001). Accordingly, cowherds might also be prone to *H. pylori* infection, while studies have scarcely addressed this issue. Thus, we aimed to investigate the prevalence of *H. pylori* infection in cowherds of Shahrekord province.

Materials and Methods

- **Study design.** In this cross-sectional study, conducted from June to October 2016, participants were randomly selected from cowherds and their neighbors living in Shahrekord province, who did not have direct contact with cows. The cows were Holstein milking cows with mean weight of 600 kg and mean age of 4 years and Simmental meat cows with mean weight of 720 kg and mean age of 2 years.

The protocol of the study was approved by Ethics Committee of Shahrekord University of Medical Sciences (Ethics code: 85-6-16). Based on the calculated sample size, 80 cowherds with at least three days a week involvement with cows were selected as the case group. They were compared with a control group selected from neighbors of the case group with no direct contact with cows and were matched for age and sex with the case group. The participants were included into the study through simple randomization method.

The design and objectives of the study were explained to all participants and written informed consent was obtained from those who were willing to participate in the study and they were ensured that their information would be kept confidential and analyzed anonymously.

Demographic information, including age, sex, job experience, educational level, number of family members, history of cancer or peptic ulcer, gastric symptoms, including epigastric pain, nausea/vomiting, and heartburn were collected. Five cc venous blood sample was taken from all participants through the left cubital vein, which was centrifuged and the separated serum was kept at -20°C and were sent to the Laboratory of Shahrekord University for ELISA in less than half an hour. The presence of *H. pylori* (Monobind Company, USA) was then compared between the groups.

In this method, calibrators were vortex and serum was diluted into 1:21 by Serum Diluent (10+200 microliter S.D), then a washing solution

was diluted with 19 volume of distilled water. 100 micro liters of calibrator and diluted serum of patients were poured into the wells. Then the wells were incubated at room temperature (21-25oC) for 30 minutes. After this time the contents of all the wells were emptied and washed 3 times with diluted washing solution, then, 100 micro liters of conjugated enzyme was added to the wells and were re-incubated and rewashed. After washing, 100 micro liters of chromogen (TMB) was added to all wells. Then, the wells were incubated at room temperature (21-25oC) for 10 minutes and 100 micro liter of the stopping solution was added to all wells and optical density of the resulting color was read at 450 nm.

Statistical Analysis

Results were presented as mean \pm standard deviation (SD) for quantitative variables and were summarized by frequency (percentage) for categorical variables. T test, chi-square and Fisher's exact tests were used for comparison of variables. For the statistical analysis, the statistical software SPSS version 21.0 for windows (SPSS Inc., Chicago, IL) was used. P values of 0.05 or less were considered statistically significant.

Results

Mean \pm SD age of the participants was 35.8 ± 14.3 (range: 14-70) years, which was not statistically different between groups ($P > 0.05$). Among all participants, only 4 cases (2.5%) were female and the rest were male ($P > 0.05$). educational level of participants was as follows: 15 participants (9.4%) were illiterate, 20 cases (12.5%) had primary school education, 29 cases (24.4%) secondary school education, 6 cases (3.8%) had high school education, 65 cases (30.6%) were high school graduates, and 15 cases (9.4%) had academic education. There were no statistically significant difference regarding educational level between two groups ($P > 0.05$). Regarding marital status, 103 participants (64.4%) were married and the rest were single, with no statistically significant difference between two groups ($P > 0.05$).

Mean \pm SD number of family members was 5.3 ± 2.1 (range: 2-10) persons with no statistically significant difference between two groups ($P > 0.05$). History of gastric cancer was observed in none of the participants.

Serum examination of H. pylori showed positive IgG in 89 participants (55.6%), which was 57.5% in the case group and 53.8% in the control group [Table 1], which was not statistically different between groups ($P > 0.05$).

Table 1. Prevalence of Anti H. pylori IgG in the participants

Anti H. pylori IgG	Positive, N	Negative, N	Total
Cowherds (case group)	46	34	80
Non-cowherds (control group)	43	37	80
Total	89	71	160

The prevalence of gastric symptoms is demonstrated in [Table 2], but prevalence of gastric symptoms was not statistically significant different between the groups and there was also no association between presences of H. pylori and gastric symptoms ($P > 0.05$). Likewise, there

were no statistically significant difference between positive H. pylori IgG and demographic characteristics of participants, including age, sex, job, educational level, marital status, and number of family members ($P > 0.05$).

Table 2. Prevalence of gastric symptoms in the participants

Gastric symptoms	Cowherds (case group), N	Non-cowherds (control group), N	Total
Gastric pain	7	11	18
Nausea	1	2	3
Heartburn	11	15	26
History of gastric ulcer	0	2	2
Gastric distension and belching	8	13	21

Discussion and Conclusion

We found no statistically significant difference between *H. pylori* IgG serology in cowherds and the control group and showed no association between positive *H. pylori* serology and number of family members, educational level, or marital status.

To the best of our knowledge, previous studies have not addressed the prevalence of *H. pylori* in cowherds to be able to compare the results with the present study. Nevertheless, some studies have selected shepherds as the target study population and have identified 100% infection with *H. pylori* in shepherds with high prevalence in their family members, which have suggested the zoonotic origin of *H. pylori* (21, 22). As long as *H. pylori* has been found in milk (18), feces (19), and gastric specimens (20) of both cows and sheep, we have hypothesized that such an association would have also been observed in cowherds. But the results of the current study indicated same prevalence between cowherds and their neighbors. The difference between the results of above-mentioned studies with the present study may be due to the improved hygienic conditions in the cowherds investigated in the present study, as the above-mentioned studies were conducted in years 2001-2003, when hygiene might have been lower and direct contact with feces and gastric liquid might have been more. In addition, a recent Iranian study has identified a high DNA sequence homology between *H. pylori* strains of sheep and human, while this homology was low between cows and humans (20), which might also justify the difference between studies on shepherds and the

present study on cowherds. Therefore, it can be concluded that sheep, but not cows, can transmit *H. pylori* to humans.

In a study in Shahrekord, the same province as the present study, 27% of the serum specimens of cows, 40% of feces, and 16% of cows' milk were positive for *H. pylori* (19). Thus, the prevalence does not seem to be high in Shahrekord cows, which might have been the reason for low prevalence of *H. pylori* in cowherds of Shahrekord, although we have not evaluated the contamination of cow products in the present study. Furthermore, some researchers have suggested that beef products are not a means of *H. pylori* transmission to humans (23), which is in line with the results of the present study, indicating no difference in *H. pylori* prevalence between cowherds and the control group.

In addition, some Iranian studies have suggested that raw cow milk might be a reservoir of *H. pylori* (19, 20, 24), which were confirmed by studies in other countries (25, 26), while other studies have determined that boiled cow milk have preventive effects against *Helicobacter* species and other food-borne pathogens (27, 28). In the present study, the neighbors of the cowherds (the control group) might have used the same cow milk as the cowherds that might have resulted in lack of significant difference between the cowherds and the control group in the present study. Moreover, nutritional status and life style, identified as risk factors of *H. pylori* infection (4), were possibly similar in two groups studied in the current study. In addition, the total prevalence in the present study was lower than

the studies on shepherds (21, 22) and was close to the total prevalence of *H. pylori* in Iran (3, 4), which shows the consistency of the results with the general prevalence of *H. pylori* in Iran.

Another finding in the present study was lack of association of demographic factors, number of family members, and gastric symptoms with the prevalence of *H. pylori*, which is similar to previous studies, indicating no associations between the prevalence of *H. pylori* and sex, type of dwelling, income, or the number of people living in the home (2), while female sex and larger family size, level of education, and hygiene were suggested as key risk factors in other studies (11, 29, 30). This difference among studies shows that the risk factors of *H. pylori* is still a controversial issue that requires further studies.

The present study included numerous strengths, including comparing prevalence of *H. pylori* in cowherds with a completely matched control group, which minimized the effect of confounding factors on the results of the study. In addition, the similarity between the prevalence of *H. pylori* with the total prevalence in Iran (3, 4) shows the reliability of the data. Furthermore, the test used to identify *H. pylori* in the present study has been introduced as a sensitive method (4) in previous studies for diagnosis of *H. pylori*, although invasive methods have higher accuracy. Nevertheless, the present study also included some limitations, including lack of assessing cows' milk and gastric specimens to determine the prevalence of *H. pylori* in cows' products. Future studies including a large number of cowherds with simultaneous determination of prevalence of *H. pylori* in cows' products can add to the results of the present study, in conclusion, prevalence of *H. pylori* in cowherds was similar to the control group, suggesting that cows are not a means of transmission of *H. pylori* to humans.

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