Isolation and identification of Helicobacter pylori from raw chicken meat in Dhamar Governorate, Yemen

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Abstract

Although Helicobacter pylori (H. pylori) is one of the most common bacterial pathogens of human, its natural reservoirs are still unclear. There is an increasing number of reports that document the occurrence of H. pylori in various foods. This study aimed at isolation of H. pylori from chicken meat sampled. Two hundred and sixty samples were collected randomly from slaughterhouses and markets in Dhamar Governorate, Yemen. Samples were enriched in Brain-Heart Infusion broth in microaerophilic conditions before inoculating the Camp-Blood agar and EYE agar plates. Results showed that 13.8% of samples were contaminated evidenced by H. pylori growth via traditional culture method on agar media. No significant differences between sample types (thighs and breast muscles) (p=0.353) or the sampling source (p=0.816) were observed. Autumn season was associated with increased occurrence of H. pylori. The source of H. pylori in food is still not identified. Proper cooking and good sanitation practices are highly recommended to avoid the infection. Further studies addressing the potential sources of H. pylori are highly suggested.

Introduction

Helicobacter pylori (H. pylori) is one of the most prevalent human bacterial pathogens globally (Sjömina et al., 2018). It is estimated that about two-thirds of the world’s population are infected with H. pylori, predominantly in developing countries with higher occurrence in poor and unhygienic areas. The prevalence of H. pylori infection depends on diverse contributing factors such as socioeconomic status, geographical area, living conditions, and personal hygiene (Sjömina et al., 2018; Al Mashhadany, 2020). Infected individuals are the main reservoir of H. pylori, however, most of these infections are asymptomatic (Denic et al., 2020). Clinically, H. pylori infection in human is associated with chronic gastritis, peptic ulceration, duodenal ulcer, gastric cancer as well as mucosa associated lymphoid malignancies (Almashhadany & Mayass, 2018; Denic et al., 2020).

From bacteriology perspective, H. pylori is ~2-3.5×0.5-1.0 μm small, curved, microaerophilic, lophotrichous gram-negative, S-shaped or curved rod bacterium. It has copious amounts of urease enzyme to survive the acidic environment of the stomach by converting urea to ammonia. The production of ammonia around H. pylori neutralizes the acidity of the stomach, making it more hospitable for H. pylori. Moreover, the helical shape of H. pylori allows it to be hidden in the mucus layer which is less acidic than the surface or the lumen of the stomach (Saedi & Sheikhshahrokh, 2016; Al-Mashhadany et al., 2018).

Molecular epidemiology studies had detected H. pylori DNA in different food-stuffs, water, and animals which suggest the existence of reservoirs for H. pylori outside human gastrointestinal tract (Montaz et al., 2014; Mousavi et al., 2015). Milk, meat, and vegetables are a potential source of H. pylori infections (Duynhoven & Jonge, 2001; Herrera, 2004). Milk products are the most studied, probably because the infection is mainly acquired during childhood and milk is mostly consumed during this period (Al-Mashhadany & Mayass, 2017; Talimkhani & Mashak, 2017). Nonetheless, the role of foods as a transmission medium is not well-validated clinically. The most commonly accepted hypothesis of H. pylori transmission is the oral-fecal route (Sjömina et al., 2018). Despite the absence of solid evidence of foods as a reservoirs for H. pylori, different studies had isolated or identified different strains and raised concerns about the contribution of nonhuman sources (Keenan et al., 2010; Talimkhani & Mashak, 2017; Hamada et al., 2018). Suboptimal sanitation conditions are favored for oral-fecal & oral-oral transmission of H. pylori in institutions of disable individuals and orphanages (Sjömina et al., 2018). Several studies have reported the survival and presence of H. pylori in foods and water, particularly in ready-to-eat products and milk, proposing that they can be sources of infection (Quaglia & Dambrosio, 2018). Foods intrinsic factors, such as pH ranging (4.9 to 6.0) and water activity (>0.97) could theoretically provide good conditions for H. pylori survival. Therefore, data on survival ability may be more significant than concerns about the growth of the bacteria in foods when determining the role of different types of food in H. pylori transmission to humans (Quaglia et al., 2007; Quaglia & Dambrosio, 2018; Al-mashhadany 2018). The prevalence of H. pylori infection in Yemen is not well-defined as various studies reported a wide range of 10-82.2% (Gumaid et al. 2003, Al-Shamahy 2005, Bahumid et al. 2009, Almashhadany & Mayass 2018), and its transmission routes in poor developing countries are a matter for wide debated (Alsulaimany et al. 2020). This study was conducted to detect the occurrence of H. pylori in raw chicken meat at Dhamar Governorate (Yemen). The relationship between occurrence of H. pylori in chicken meat and months during the period of study was also addressed.
Materials and Methods

Study design and sampling

Two hundred and sixty (260) raw chicken meat samples (140 Thigh, and 120 Breast), were collected from retail markets and chicken slaughterhouses in different places of Dhamar Governorate, from July to December 2020. The samples were put in sterile cooled polyethylene bags and kept in ice box with temperature approximately 4°C during transport and storage at the laboratory (Almashhadany, 2021b). The bacteriological analysis was performed within 2 h of sample collection.

Isolation of H. pylori

In the laboratory, the isolation of H. pylori was done under aseptic conditions as previously published (Al-Mashhadany & Mayass, 2017; Almashhadany, 2021a). Briefly, samples were cut into small pieces using sterile blades for liberation of adherent bacteria. From thigh and breast, 25 gm (as an optimal sample size) was soaked in 250 ml of normal saline. For enrichment, a volume of 0.5 ml of the suspension was then placed in a 4.5-ml Brain-Heart Infusion broth with 7% horse serum without antibiotics and incubated in a microaerophilic atmosphere (GasPack; Oxoid, Basingstoke, England) at 37°C for 3 to 7 days. After that, modified Campy-bloog agar and EYE agar plates were inoculated with 100 μl of the enriched suspension and incubated at 37°C in microaerobic condition in a candle jar and Campy Gen (2.5 L) in the incubator for 4-10 days. For purification purposes, developed colonies were subcultures on the same agar media and incubated at 37°C for 48–72 hrs. (Coldham et al., 2011; Lawson, 2015).

Identification of H. pylori

Identification of H pylori isolates was done according to a published standard scheme (Lawson, 2015; Al-Mashhadany & Mayass, 2017). Briefly, after incubation, all cultural plates were examined for suspected colonies of H. pylori. Gram staining was done according to the standard protocol with exposure of smears to safranin for 3 minutes. Biochemical tests employed for the identification included: Catalase, Oxidase, Urease, Indole production, growth in 1% glycine, growth in 3.5% NaCl, H₂S production in (TSI), TSI with lead acetate paper, resistance to nalidixic acid, sensitivity to cephalothin, and hippurate hydrolysis. Isolates that met the reference characteristics were considered H. pylori (Lawson, 2015; Al-Mashhadany & Mayass, 2017).

Statistical analysis

Data were analyzed using SPSS software (version 25), confidence intervals (CI) were estimated using normal distribution approximation at an alpha level of 0.05. Chi-square test was used to evaluate differences between groups.

Results

Occurrence of H. pylori in raw chicken meat samples

From 260 raw chicken meat samples, 18 (13.8%) showed a positive result for H. pylori. This result includes 11 (15.7 %) positive samples from thigh and 7 (11.7%) positive samples from breast (Table 1). There is no significant difference between sample types in terms of contamination with H. pylori (p=0.353). Based on this sample size, up to 18% of chicken meat samples are expected to be contaminated with H. pylori.

Occurrence of H. pylori according to sampling location

Regarding to the distribution of H. pylori among examined samples, the results showed a slightly higher occurrence of H. pylori in samples from slaughterhouses (Table 2). However, this increase was not significant statistically (χ²=0.054, p=0.816).

Temporal distribution

The changes in occurrence of H. pylori were monitored throughout the study period. The highest rate of H. pylori was observed in October (23.8%) and September (22.7%), while the lowest rate was found in June (5.0%) and August (8.3%) (Figure 1). Autumn was significantly associated with increase in H. pylori contamination of chicken meat (p=0.007).

Table 1. Occurrence of H. pylori in raw chicken meat according to type of meat.

| Chicken meat | No. of samples | Positive Samples n (%) | 95% CI       |
|--------------|----------------|------------------------|--------------|
| Thigh        | 140            | 22 (15.7)              | 9.69 – 21.74 |
| Breast       | 120            | 14 (11.7)              | 5.92 – 17.41 |
| Total        | 260            | 36 (13.8)              | 9.65 – 18.04 |

Table 2. Occurrence of H. pylori in raw chicken meat according to sampling location.

| Chicken meat | Slaughterhouses | Retail markets |
|--------------|-----------------|----------------|
|              | No. of tested   | Positive samples n (%) | No. of tested | Positive samples n (%) |
| Thigh        | 80              | 12 (15.0)           | 70            | 10 (14.3) |
| Breast       | 60              | 8 (13.3)            | 50            | 6 (12.0)  |
| Total        | 140             | 20 (14.3)           | 120           | 16 (13.3) |
Discussion

The occurrence and survival of *H. pylori* in different foods have been a hot area of research during the past decades. Studies addressing the occurrence of *H. pylori* in meat are rare, whereas the majority of published literature focused on milk and milk products (Herrera 2004, Quaglia & Dambrosio 2018). Recently, stomach of domestic animals have been found to harbor high numbers of *H. pylori*, which suggests domestic animals as an important reservoir (Saedi & Sheikhshahrrokh 2016). Therefore, this study aimed to detect *H. pylori* in chicken raw meat.

Out of two hundred and sixty (260) raw chicken meat samples collected in this study, 36 (13.8%) were contaminated with *H. pylori*. This result is consistent with studies from Iran that found 10-14% of salad and vegetable samples harbored *H. pylori* (Atapoor et al. 2014, Yahaghi et al. 2014). Hemmatinezhad and associates in Iran, reported that 13.45% of ready-to-eat food samples were contaminated with *H. pylori* (Hemmatinezhad et al. 2016). Likewise, a similar occurrence was reported in raw milk detected by bacteriological culture or by molecular detection of ureC gene (Rahimi & Kheirabadi 2012, Kazemeini et al. 2014, Talaei et al. 2015). On the contrary, other studies documented higher rates (20 - 36%) in different foods including chicken raw meat (Dore et al. 2001, Meng et al. 2008, Mousavi et al. 2015, Saedi & Sheikhshahrrokh 2016). The role of food prepared under poor hygienic conditions as a possible vehicle for *H. pylori* transmission was suggested by Begue and colleagues, who found significant hazards for consumption of food obtained from street vendors in Peru (Begue et al. 1998). The actual sources of *H. pylori* in chicken raw meat have not been identified. However, contaminated water, infected handlers, and chicken gastrointestinal tract are the most probable sources (Meng et al. 2008, Vale & Vitor 2010, Quaglia & Dambrosio 2018).

Regarding the distribution of *H. pylori* among examined samples, it seems that sample type is not a contributing factor for occurrence of *H. pylori*. This observation is supported by a recent study in poultry slaughter-houses in Egypt that found 3.33% of liver samples to be contaminated with *H. pylori*, while 2.22% samples of meat and gizzard were contaminated (Hamada et al. 2018). According to our findings and previous investigations, *H. pylori* truly occur in foods (Duynhoven & Jonge 2001, Atapoor et al. 2014, Saedi & Sheikhshahrrokh 2016, Quaglia & Dambrosio 2018). However, techniques for direct isolation of *H. pylori* from foodstuff have not been fully developed or standardized. Indeed, the isolation of *H. pylori* from food products is quite difficult due to the presence of associated microflora and to the probably very low *H. pylori* load (Vale & Vitor 2010, Talimkhani & Mashak 2017).

The relationship between months and occurrence of *H. pylori* during the work in Dhamar Governorate was studied. The highest rates of isolation of *H. pylori* were found in October (23.8 %) and September (22.7 %). However, the occurrence of *H. pylori* was seen to decrease prior September and after October. This observation contradicts the previous study that did not find *H. pylori* in February, March, July, August, and September (Al-Mashhadany & Mayass 2017). In fact, the seasonality of *H. pylori* occurrence in poultry meat is still undressed. Autumn is a wet season in Dhamar that may provide favorable conditions for *H. pylori* proliferation in animals’ gastrointestinal tracts that is reflected by higher contamination observed in September and October.

Conclusions

*H. pylori* occurs in foods and may be an important media for its transmission (horizontal method). The occurrence of *H. pylori* in raw chicken meat in Dhamar Governorate seems to be high, mostly due to poor living conditions, socioeconomic status, and sanitary habits, or other risk factors. The seasonality of *H. pylori* in poultry meats is still unclear. In addition, special emphasis on proper cooking of chicken meat before consumption is recommended.

References

Al-Mashhadany DA, Mayass SM, 2017. Incidence of Helicobacter pylori in food and water in Dhamar governorate/ Yemen. Int J Curr Res 9:4520–6.

Almashhadany DA, Mayass SM, 2018. Prevalence of Helicobacter pylori in Human in Dhamar Governorate / Yemen. J Med Pharm Sci 2:1–18.

Al-mashhadany DA. 2018. Application of Stool Antigen Test for Monitoring Helicobacter pylori among Human in Erbil Governorate, Kurdistan Region, Iraq Int J Pharm Pharm Sci 10:49–3.

Al-Mashhadany DA, 2020. Epidemiology of Helicobacter pylori among Human at Erbil Governorate/Kurdistan Region / Iraq. World J Pharm Pharm Sci 9: 435–47.

Al-Shamahy HA, 2005. Seroprevalence of Helicobacter pylori among children in Sana’a, Yemen. Ann Saudi Med 25:299–03.

Almashhadany DA, 2021a. Impact of heat treatment on the antimicrobial residues in raw goat’s milk. Iraqi J Vet Sci 35:549–3.

Almashhadany DA, 2021b. Diagnosis of brucellosis in sheep and goats raw milk by fast and reliable techniques. Iraqi J Vet Sci 35:663–8.

Al-Mashhadany DA, Ismael LQ, Zaki AM, 2018. Seroprevalence of Helicobacter pylori among human in Erbil governorate, Kurdistan region, Iraq Res J Life Sci Bioinform Pharm Chem Sci 4:268–80.

Alsulasimany FAS, Awon ZA, Alamhahy AM, Koumi MI, Yaghmoor BE, Elhady SS, Elfaky MA, 2020. Prevalence of Helicobacter pylori Infection and Diagnostic Methods in the Middle East and North Africa Region. Medicina (B. Aires). 56:169.

Atapoor S, Dekhordi FS, Rahimi E, 2014. Detection of Helicobacter pylori in Various Types of Vegetables and Salads. Jundishapur J Microbiol 7:10013.

Bahumid NM, Al-Kazimi G, Tares AS, Abdullah HS, Khamis I, 2009. An epidemiological view of Helicobacter pylori infection among patients who underwent upper gastrointestinal tract endoscopy in Aden. Univ Aden J Nat Appl Sci 13:159–64.

Begue RE, Gonzales JL, Correa-Gracian H, Tang S, 1998. Dietary risk factors associated with the transmission of Helicobacter pylori in Lima, Peru. Am J Trop Med Hyg 59:637–40.

Coldham T, Rose K, O’rourke J, Neilian BA, Dalton H, Lee A, Mitchell H, 2011. Detection, isolation, and characterization of Helicobacter species from the gastrointestinal tract of the brushtail possum. Appl environ microbiol 77:1581-7.

Denic M, Touati E, Reuse H De, 2020. Pathogenesis of Helicobacter pylori infection. Helicobacter 25:e12736. DOI:10.1111/HEL.12736.

Dore MP, Sepulveda AR, El-Zimaty H, Yamaoka Y, Osato MS, Mototsugu K, Nieddu AM, Realdi G, Graham DY, Denic M, Touati E, Reuse H De, 2020. Pathogenesis of Helicobacter pylori infection. Helicobacter 25:e12736. DOI:10.1111/HEL.12736.

Dore MM, Sepulveda AR, El-Zimaty H, Yamaoka Y, Osato MS, Mototsugu K, Nieddu AM, Realdi G, Graham DY, Denic M, Touati E, Reuse H De, 2020. Pathogenesis of Helicobacter pylori infection. Helicobacter 25:e12736. DOI:10.1111/HEL.12736.

Duynhoven YTHP van & Jonge R de, 2001. Transmission of Helicobacter pylori: a role for food? Bull World Health Organ 79: 455–60.

Gunaid AA, Hassan NA, Murray-Lyon I, 2003. Prevalence and risk factors for...
Helicobacter pylori infection among dyspeptic patients. Saudi Med J 24:512–7.

Hamada M, Elbehiry A, Marzouk E, Moussa IM, Hessain AM, Alhaji JH, Heme HA, Zahran R, & Abdeen E, 2018. Helicobacter pylori in a poultry slaughterhouse: Prevalence, genotyping and antibiotic resistance pattern. Saudi J Biol Sci 25:1072–8.

Hemmatinezhad B, Momtaz H, Rahimi E, 2016. 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 15:1–9.

Herrera AG, 2004. Helicobacter pylori and Food Products: A Public Health Problem. In: Spencer JFT, de Spencer RAL, eds. Public Health Microbiology: Methods and Protocols. Humana Press Inc, Totowa, NJ. pp. 297–301.

Kazemeini H, Rahimi E, Kianpour F, 2014. Prevalence of Helicobacter pylori in buffalo milk in Iran. Iran J Public Health 43:174.

Keenan JI, Salm N, Hampton MB, Wallace AJ, 2010. Individual and combined effects of foods on Helicobacter pylori growth. Phyther Res 24: 1229–33.

Lawson AJ, 2015. Helicobacter. In: Jorgensen JH, Pfaller MA, eds. Manual of Clinical Microbiology 11th ed., John Wiley & Sons, Ltd., New York, NY, pp 1013–1027.

Meng X, Zhang H, Law J, Tsang R, Tsang T, 2008. Detection of Helicobacter pylori from food sources by a novel multiplex PCR assay. J. Food Saf. 28: 609–619.

Momtaz H, Dabiri H, Souod N, Gholami M, 2014. Study of Helicobacter pylori genotype status in cows, sheep, goats and human beings. BMC Gastroenterol 14:1–7.

Mousavi S, Dehkordi FS, Rahimi E, 2015. 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 20:1–7.

Quaglia NC, Dambrosio A, 2018. Helicobacter pylori: A foodborne pathogen? World J Gastroenterol 24:3472.

Quaglia NC, Dambrosio A, Normanno G, Parisi A, Firinu A, Lorusso V, Celano GV, 2007. Survival of Helicobacter pylori in artificially contaminated ultrahigh temperature and pasteurized milk. Food Microbiol 24:296–300.

Rahimi E, Kheirabadi EK, 2012. Detection of Helicobacter pylori in Bovine, Buffalo, Camel, Ovine, and Caprine Milk in Iran. Foodborne Pathog Dis 9:453–6.

Saeidi E, Sheikhshahrokha A, 2016. VacA genotype status of Helicobacter pylori isolated from foods with animal origin. Biomed Res Int 2016:1–6.

Sjomina O, Pavlova J, Niv Y, Leja M, 2018. Epidemiology of Helicobacter pylori infection. Helicobacter 23:e12514.

Talaee R, Souod N, Momtaz H, Dabiri H, 2015. Milk of livestock as a possible transmission route of Helicobacter pylori infection. Gastroenterol Hepatol from Bed to Bench 8: S30.

Talimkhani A, Mashak Z, 2017. Prevalence and Genotyping of Helicobacter pylori Isolated From Meat, Milk and Vegetable in Iran. Jundishapur J Microbiol 2017:10.

Vale FF, Vitor JMB, 2010. Transmission pathway of Helicobacter pylori: Does food play a role in rural and urban areas? Int J Food Microbiol 138:1–12.

Yahaghi E, Khamesipour F, Mashayekhi F, Dehkordi FS, Sakhaei MH, Masoudimanesh M, Khameneie MK, 2014. Helicobacter pylori in vegetables and salads: Genotyping and antimicrobial resistance properties. Biomed Res Int 2014.