Ready-to-eat foods as a possible source of *Helicobacter pylori*

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Abstract

Ready-to-eat (RTE) foods can be risky for consumers as they are not usually cooked or heated before consumption. We set out to assess RTE foods available in the Czech market network as a possible source of *Helicobacter pylori*. Attention was paid to RTE foods containing at least one ingredient of animal origin. A total of 50 food samples were chosen and divided into 3 categories. The "composite foods" category (n = 29) included sandwiches (n = 5), baguettes (n = 11), tortillas (n = 6), buns (n = 2), toast bread (n = 1), croissant (n = 1), bagels (n = 2), and pretzel (n = 1). The "salads" category included commercially produced mixed salads (n = 13). The "other" category (n = 8) included smoked salmon (n = 2), smoked mackerel (n = 2), smoked herring (n = 1), marinated salmon (n = 1), surimi (n = 1) and carpaccio (n = 1). The samples were analysed using nested-PCR. The *glmM* gene of *H. pylori* was detected in 50% (n = 25) of all samples. The "composite foods" category had the highest number of positive samples 58.6% (n = 21), followed by "other" with 50% (n = 4), and "salads" with 30.8% (n = 4). The results of our study showed that consumers eating RTE foods may be exposed to a risk of infection with *H. pylori*.

Food safety, microbiological risk, nested-PCR

Helicobacter pylori infection remains a major public health problem worldwide. Based on a survey of data in a systematic review published by Hooi et al. (2017), it is estimated that approximately 4.4 billion people worldwide were *H. pylori* positive in 2015. The prevalence of *H. pylori* infection varies widely between regions and countries. These differences in incidence likely reflect the level of urbanization, sanitation, access to drinking water, and different socioeconomic status of the population.

The mode of transmission of *H. pylori* has not yet been properly explained and has become a subject of debate. Much of the scientific literature points towards interpersonal transmission, with two main transmission models – vertical transmission from parents to children within the same family through direct personal contact (gastro-oral, oral-oral or faecal-oral routes), and horizontal transmission between non-family members. Some authors are of the view that infection may occur through multiple routes rather than a single route (Goodman and Correa 1995; Oderda 1999; Velázquez and Feirtag 1999; Miyaji et al. 2000; Vale and Vítor 2010). Over the past two decades, the role of *H. pylori* as a potential pathogen in human and veterinary medicine has been intensively investigated, and the results of some studies suggest possible zoonotic transmission. The reservoirs of *H. pylori* are mainly livestock such as cattle, buffaloes, and sheep, which has been confirmed by some studies (El-Gohary et al. 2015; Talaei et al. 2015; Guessoum et al. 2018). Meng and Doyle (1997) proposed the hypothesis that food may be an important vehicle for *H. pylori* transmission. Since then, the presence of *H. pylori* has been shown in a variety of foods of animal and plant origin such as milk and dairy products, meat, raw vegetables, salads, and fish (Fujimura et al. 2002; Zamani et al. 2017; Quaglia and Dambrosio 2018). An important aspect in the transmission of *H. pylori* via contaminated food is its ability to survive in food under certain conditions. Published data show that once inoculated, *H. pylori* can survive for varying lengths of time in foods such as milk,

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vegetables, meat, and dairy products (Zamani et al. 2017) and even in drinking water (Fan et al. 1998).

Currently, there are no standardised culture methods available to guarantee the isolation of *H. pylori* from specific samples like foodstuff. The main disadvantage consists in the inability to capture the coccoid, viable non-culturable form (VNC) of the bacterium. Food samples are also rich in accompanying microbiota, which can make the growth of *H. pylori* on agar significantly indisposed. On the other hand, the nested-PCR approach allows to overcome the constraints of the culture method (Quaglia and Dambrosio 2018; Furmančíková et al. 2022). Although PCR based methods do not discriminate between living or dead microorganisms, they appear to be very helpful as a screening method to determine possible contamination of samples. Yet even this disadvantage already has a solution in the form of the implementation of special dyes (e.g., ethidium monoazide) which enable the distinction between living and dead cells. The principle lies in the different permeability of the dye through the cell wall of dead and living bacteria (including VNC) and its binding to DNA, which is then incapable of amplification (Liang et al. 2022).

Ready-to-eat (RTE) foods may be the source of *H. pylori* as has been confirmed by several studies (Meng et al. 2008; Ghorbani et al. 2016; Hemmatinezhad et al. 2016). RTE foods are defined as foods that are intended for direct human consumption without the need for cooking or other processing that can effectively remove or reduce the load of microorganisms of concern to acceptable levels (Commission Regulation EC 2073/2005). As they are not heated or cooked before consumption, RTE foods may pose a potential risk to consumers. The microbiological risk is mainly due to their possible contamination during production, preparation, and storage. Therefore, increased attention needs to be paid with regard to the microbiological risks associated with their consumption. The safety of RTE foods depends on the quality of the raw materials, the technological process of production and other factors (type and composition of food, storage temperature, type of packaging, shelf life, hygiene of workers and equipment, and cross-contamination). It is hence essential to follow good hygiene and manufacturing practices in the production of RTE foods to avoid contamination of products during their production (Gurler et al. 2015; Kotzekidou 2016; Mengistu and Tolera 2020). The aim of this study was to assess whether commercially available RTE foods can be a potential source of *H. pylori*. Specific attention was paid to RTE foods containing processed products of animal origin.

Materials and Methods

Materials

A total of 50 RTE foods were analysed in this study. The samples were purchased from retail stores in the Czech Republic – 4 large supermarket chains and two counter stalls. Sampling was carried out in accordance with the ISO standard (ISO/TS 17728, 2017). The samples obtained from the supermarket chains (n = 46) were packaged and were on sale on the shelves. Samples obtained from counter stalls (n = 4) were on sale without any packaging. Table 3 shows the distribution of RTE foods based on the packaging method. RTE foods were selected with an emphasis on their composition. One criterion was at least one ingredient being a processed product of animal origin. The temperature was maintained between 4–6 °C during transport until being stored in the laboratory. Laboratory testing was carried out within 2 h of purchase from the market. The samples were divided into 3 categories according to the type of RTE food (Table 1). Samples in the "composite foods" category were made using a wide range of foods. Among the foods of animal origin used in the production of these sandwiches were fish (salmon, tuna), cooked pork or chicken, other meat products, cooked eggs, and cheese. In addition to foods of animal origin, products in this category also contained fresh, preserved, or cooked vegetables, spreads and dressings. The "salads" category included products that contained foods both of animal and plant origin. They contained 28.5–65.0% fresh vegetables (one or more types of lettuce and other vegetables), or preserved vegetables, dried vegetables, and foods of animal origin (cheese, fish, smoked, roasted or grilled chicken, other meat products) and various dressings. The "other" category were all single-ingredient products.

Methods

Bacterial DNA was isolated from RTE food samples using the NucleoSpin Microbial DNA kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. Detection of *H. pylori* was performed by

nested-PCR aimed at the detection of the *glmM* gene of *H. pylori.* The reaction mixes used for *glmM* detection (Furmančíková et al. 2022) were prepared with 12.5 µl of PPP Master mix (Top-Bio s.r.o., Vestec, Czech Republic), 8.0 µl of PCR H_2O (Top-Bio s.r.o., Vestec, Czech Republic) and 0.5 µM µmol/l of oligonucleotide primers [Hp1,2 – first reaction; Hp3,4 – second reaction (Quaglia et al 2009), 1.25 µl of Hp1 and Hp2, respectively (0.5 µM µmol/l)]. Primers were synthesized by Generi Biotech s.r.o. (Hradec Králové, Czech Republic). Two µl of extracted DNA were added to 23 µl of the first reaction mixture, and 2 µl of the final product from the first reaction were added to 23 µl of the second reaction mixture. The nested-PCR method was performed according to Quaglia et al. (2009). The samples were cooled to 6 °C. Electrophoretic separation was performed on a 1.5% agarose gel stained with Midory Green Advance (Nippon Genetics Europe, Düren, Germany), followed by visualization of the PCR product of about 252 bp under UV light. The electrophoresis was performed at 120 V, 90 mA for 60 min. DNA Ladder of 100–4000 bp (Lonza Rockland, Inc., Rockland, USA) was used as a marker. The positive control was *H. pylori* DNA from a strain obtained from a human gastric mucosal sample.

Results

A total of 50 RTE food samples were examined in the present study. The set of samples was divided into 3 categories. The first category consisted of composite foods samples, the second category comprised salads and the "other" category included 3 different product groups – surimi, carpaccio, and fish products (Table 1).

Table 1. Distribution of ready-to-eat food samples into categories with the number of samples.

Food category	Type of product	Number of samples in the type of product	Number of samples in the food category
Composite foods	Sandwich	5	29
	Baguette	11	
	Wrap	4	
	Tortilla	2	
	Bun	2	
	Toast	1	
	Croissant	1	
	Bagel	2	
	Pretzel	1	
Salads	Mixed salads	13	13
Other	Smoked salmon	2	8
	Smoked mackerel	2	
	Smoked herring	1	
	Surimi	1	
	Marinated salmon	1	
	Carpaccio	1	

The samples were analysed using nested-PCR. Of the total number of samples tested, the presence of the *glmM* gene, the detection of which clearly confirms the presence of *H. pylori*, was found in 50% of the samples (n = 25). The number of positive samples in each category and type of product is shown in Table 2. The highest number of positive samples, 58.6% (n = 21), was found in the "composite foods" category. A total of 30.8% of the salad samples tested positive (prosciutto salad with mustard dressing n = 1; spicy salad with chicken and dressing n = 1; salad with chicken and rocket n = 1; mixed salad with goat cheese, beetroot, and walnuts n = 1). In the "other" category, 50% of the samples showed the presence of *H. pylori* (beef carpaccio n = 1; surimi n = 1; smoked mackerel n = 1; salmon gravlax with honey mustard sauce n = 1). The occurrence of *H. pylori* was recorded mainly in protected atmosphere packaged types of RTE foods (Table 3).

Table 2. Positive samples in each category and type of ready-to-eat food.

Food category	Type of product	Number of samples examined	Number of positive samples
Composite foods	Sandwich	5	2
	Baguette	11	7
	Wrap/Tortilla	6	3
	Bun	2	2
	Toast	1	0
	Croissant	1	1
	Bagel	2	1
	Pretzel	1	1
Salads	Mixed salad	13	4
Other	Smoked salmon	2	0
	Smoked mackerel	2	1
	Smoked herring	1	0
	Carpaccio	1	1
	Surimi	1	1
	Marinated salmon	1	1

Table 3. The number of *H. pylori* positive findings depending on the type of RTE food packaging.

Purchased	Total number of samples	Number of samples according to packaging type		Number of positive samples
Counter stalls	4	No packaging	4	4
Supermarkets	46	Vacuum	7	3
		Protected atmosphere	36	17
		Simple	3	1

Discussion

The range of RTE foods varies from country to country, depending on the dietary habits of local consumers, the availability of RTE foods at the point of production, and the available refrigeration options during production, storage, and distribution. RTE foods can include a wide variety of different foods that can be raw, cooked or otherwise processed, and can be sold hot or chilled. The range of RTE foods is highly diverse, from single-ingredient foods to complex meals (Kotzekidou 2016; Mengistu and Tolera 2020; Mengistu et al. 2022). The most frequent occurrence of *H. pylori* was detected in food packaged in a protected atmosphere. This finding is in accordance with the requirements of *H. pylori* for a specific environment, especially a microaerophilic atmosphere, in which it can survive for a long time (Quaglia and Dambrosio 2018).

The frequency of positive samples is higher compared to the results obtained by Meng et al. (2008), who found the presence of *H. pylori* in 44% of RTE food samples tested by multiplex PCR. In the study, 18 samples of raw tuna (*Thunnus thynnus)* included in sushi were collected from two restaurants in Chicago, Illinois, USA. The authors concluded that the fish had been contaminated with *H. pylori* probably during processing. The likely source of the fish contamination could also have been workers who come into contact with RTE food during processing, or via cross-contamination by inadequately cleaned tools or

utensils used during food preparation. Ghorbani et al. (2016) found a much lower percentage (20%, n = 60) of positive RTE food samples in their Iranian study that included a total of 300 samples (vegetable, poultry and other meat sandwiches, ham, fish). The samples were analysed using the culture method. Positive samples were then subjected to PCR analysis to detect the *vacA* genotype. The RTE food types with the highest prevalence of positive samples were sandwiches with vegetables (45%), minced meat (32%), and meat sandwich (20%). Given the presence of similar genotypes of *H. pylori* strains in foods as in human biopsy samples, the authors concluded that contaminated foods may be the source of bacteria for humans.

All samples tested contained food of animal origin as one of the ingredients. According to literature data, meat and meat products may be a vector of *H. pylori*. Ghorbani et al. (2016) detected the presence of *H. pylori* in ham samples (8.3%) and in minced meat samples (32%) using the culture method. The presence of *H. pylori* was also detected in poultry meat. Meng et al. (2008) examined fresh poultry meat samples (n = 11) collected from a grocery store. Thirty-six percent of the chicken samples (n = 4) were *H. pylori* positive. In contrast, some studies did not confirm the presence of *H. pylori* in meat or meat products. When Hemmatinezhad et al. (2016) investigated the prevalence of *H. pylori* in different types of RTE foods including those containing meat products, *H. pylori* was not detected in any of the 50 salami and 50 sausage samples tested by multiplex PCR.

A significant risk in ensuring the safety of RTE foods is the fact that *H. pylori* is able to survive for a certain period of time in food under certain conditions, as demonstrated by a number of studies (Stevenson et al. 2000; Gomes and De Martinis 2004; Buck and Oliver 2010). The ability of *H. pylori* to survive in meat and meat products has been confirmed (Poms and Tatini 2001). Meat and meat products create an ideal environment for the growth and survival of bacteria, including *H. pylori,* given their composition (salt concentration, abundance of amino acids) and physicochemical parameters (optimal a_w and pH values). Stevenson et al. (2000) set out to determine how long *H. pylori* can survive in chilled or frozen ground beef. The results have shown that although the microorganism dies rapidly in inoculated ground beef, whether chilled or frozen, it is able to survive for some time. The counts of *H. pylori* in chilled samples decreased from an initial 3.3 $\log_{10}$ CFU/g on day 0 to 1.4 $\log_{10}$ CFU/g on day 6. When samples were frozen, *H. pylori* levels decreased even more rapidly, from 3.3 $\log_{10}$ CFU/g on day 0 to 0.5 $\log_{10}$ CFU/g on day 6.

Other foods of animal origin that were used in the production of some of the RTE foods analysed (sandwiches and salads) were dairy products (cheese). Out of a total of 17 positive samples in the "composite foods" category, dairy products were included in 4 samples. These included semi-hard and hard cheeses such as Gouda, Emmental, or Cheddar. In the "salads" category, goat cheese was an ingredient in 1 of the 4 positive samples. Some studies have shown that even dairy products can be a source of *H. pylori*. Mousavi et al. (2014) studied the occurrence of *H. pylori* in traditional Iranian dairy products. They analysed cheese, cream, butter and ice cream made from unpasteurized milk purchased from supermarkets. Cheese was among the most contaminated dairy products (30% of positive samples). Khaji et al. (2017) and Esmaeiligoudarzi et al. (2015) focused on the detection of *H. pylori* in dairy products. Khaji et al. (2017) analysed 220 samples of different dairy products using the culture method followed by PCR amplification of the 16s rRNA gene. A total of 29 (13.2%) of the samples tested were positive. Esmaeiligoudarzi et al. (2015) detected *H. pylori* in cheese and cream samples using the culture method followed by PCR confirmation by detecting the *ureC* gene. Out of a total of 120 samples, *H. pylori* was present in 10.8% of the samples. A lower percentage of positive samples (3.9%) was detected by the culture method in the study by Ranjbar et al. (2019). The sample set in this study consisted of yoghurt, butter, cream and cheese samples. These authors all agree on the important role of milk and dairy products in the transmission of *H. pylori* to humans.

Helicobacter pylori can be transmitted through contaminated vegetables, and foods containing them. The ability of *H. pylori* to survive in artificially contaminated vegetables has been shown in a number of studies (Gomes and De Martinis 2004; Atapoor et al. 2014; Ghorbani et al. 2016; Hemmatinezhad et al. 2016; Ng et al. 2017).

Compared to the results of Hemmatinezhad et al. (2016), our study found a similar percentage of positive samples in the salad category (30.8%). Hemmatinezhad et al. (2016) found the highest percentage of positive samples in the olvie salad (36%), salads from restaurants (30%), and fruit salads (28%).

Literature data show that consumption of RTE raw salads and vegetables poses a distinct immediate risk of *H. pylori* infection. One possible explanation for the high prevalence of *H. pylori* in RTE foods containing vegetables is the inadequate washing of vegetables during their preparation. Vegetables are often grown in soil to which animal faeces are applied as fertilizer. The importance of the role of animal faeces in the transmission of *H. pylori* was described by Ho et al. (1991). Kotzekidou (2016) reported that irrigation water quality and the type of irrigation system significantly affect the microbial safety of fresh produce (fresh fruits and vegetables). Flood and spray irrigation pose the greatest risk because contaminated water is applied directly to edible crop leaves. If the crop is irrigated with contaminated water prior to harvest, or if cross-contamination with cutting tools occurs during the harvesting process, human pathogens can be relocated into internal plant tissue.

Another possible explanation for the high prevalence of *H. pylori* in vegetables is their high-water activity and optimal pH. Finally, infected food handlers in food processing plants may play a role in the contamination of vegetable foods (Ghorbani et al. 2016). Ng et al. (2017) demonstrated that the ability of *H. pylori* to form biofilms and microcolonies plays an important role in the survival of *H. pylori* on the surface of vegetables. They reported that *H. pylori* can form a biofilm in conjunction with other contaminating microbiota, which helps prolong its survival time on the surface of vegetables. Due to the difficulty of eliminating biofilm in the normal process of washing vegetables, it is important to follow good hygiene and production practices when producing vegetables and their products. The results of the study confirmed that *H. pylori* is able to survive in extragastric environments, supporting the theory that water and food can be the source and a possible route of *H. pylori* transmission (Ng et al. 2017).

A study was conducted in Iran to detect *H. pylori* in different types of traditional and commercial salads and in samples of washed and unwashed vegetables (Atapoor et al. 2014). The study showed *H. pylori* contamination in different vegetables (basil, spinach, lettuce, parsley, leek, radish). The results showed that 44 out of 460 samples examined (9.56%) were positive for *H. pylori* by the culture method. Unwashed leeks (35%) were the most commonly contaminated vegetables, followed by unwashed basil (25%) and unwashed lettuce (20%). According to the authors, vegetables are a source of *H. pylori* and one way to reduce contamination of vegetables is to wash them thoroughly (Atapoor et al. 2014).

In the "other" category, 50% of the samples showed the presence of *H. pylori.* Both Meng et al. (2008) and Ghorbani et al. (2016) demonstrated the presence of *H. pylori* in raw tuna meat and RTE fish samples. One of the samples tested was a sample of carpaccio (uncooked meat product), which was found to contain *H. pylori*. Stevenson et al. (2000) investigated raw beef samples (n = 20) purchased from a retail store chain in Texas, USA; *H. pylori* was not isolated from any of the samples collected.

In conclusion, RTE foods which do not require further heat treatment before consumption and may provide suitable conditions for bacterial survival, appear to be a high-risk food group with regard to the transmission of *H. pylori.* The results of this study complement a number of different studies that have already been carried out

in geographically different territories. Certain typical specificities, such as the climate, soil composition, environmental contamination, methods of raising livestock, processing of raw materials, production processes, etc., vary among different states or regions. These factors can significantly affect the quality of the final product and thus lead to different resulting values. Therefore, mapping every other part of the world is important mainly to get a comprehensive view of the prevalence of *H. pylori* in various types of food worldwide, to be aware of the factors that influence this occurrence and to what extent. Using a sufficiently extensive collection of data, it is then possible to obtain comprehensive information for an overall evaluation of the objective degree of risk of transmission of this bacterium from food to the human population. This assessment, both on an international and local scale, may result for example in creating the foundation for a proposal to legislative changes in order to incorporate requirements for the determination of *H. pylori* in selected types of food as a regular part of the health safety control in food plants and the market network.

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References

Atapoor S, Safarpoor Dehkordi F, Rahimi E 2014: Detection of *Helicobacter pylori* in various types of vegetables and salads. Jundishapur J Microbiol **7**: e10013

Buck A, Oliver JD 2010: Survival of spinach-associated *Helicobacter pylori* in the viable but nonculturable state. Food Control **21**: 1150-1154

El-Gohary AH, Yousef MA, Mohamed AA, El-Amaiem WEA, Abdel-Kareem LM 2015: epidemiological study on *H. pylori* in cattle and its milk with special reference to its zoonotic importance. Biol Med (Aligarh) **7**: 251

Esmaeiligoudarzi D, Tameshkel FS, Arsalani M, Sohani MH, Behnod V, Ajdarkosh H 2015: Prevalence of *Helicobacter pylori* in Iranian milk and dairy products using culture and ureC based-PCR techniques. Biomed Pharmacol J **8**: 179-183

Fan XG, Chua A, Li TG, Zeng QS 1998: Survival of *Helicobacter pylori* in milk and tap water. J Gastroenterol Hepatol **13**: 1096-1098

Furmančíková P, Šťástková Z, Navrátilová P, Bednářová I, Dvořáčková M 2022: Prevalence and detection of *Helicobacter pylori* in raw cows' and goats' milk in selected farms in the Czech Republic. J Food Nutr Res **61**: 192-197

Fujimura S, Kawamura T, Kato S, Tateno H, Watanabe A 2002: Detection of *Helicobacter pylori* in cow's milk. Lett Appl Microbiol **35**: 504-507

Ghorbani F, Gheisari E, Dehkordi FS 2016: Genotyping of *vacA* alleles of *Helicobacter pylori* strains recovered from some Iranian food items. Trop J Pharm Res **15**: 1631-1636

Gomes BC, De Martinis ECP 2004: Fate of *Helicobacter pylori* artificially inoculated in lettuce and carrot samples. Braz J Microbiol **35**: 145-150

Goodman KJ, Correa P 1995: The transmission of *Helicobacter pylori.* A critical review of the evidence. Int J Epidemiol **24**: 875-887

Guessoum M, Guechi Z, Adnane M 2018: First-time serological and molecular detection of *Helicobacter pylori* in milk from Algerian local-breed cows. Vet World **11**: 1326-1330

Gurler Z, Pamuk S, Yildirim Y, Ertas N 2015: The microbiological quality of ready-to-eat salads in Turkey: A focus on *Salmonella* spp. and *Listeria monocytogenes*. Int J Food Microbiol **196**: 79-83

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

Ho SA, Hoyle JA, Lewis FA, Secker AD, Cross D, Mapstone NP, Dixon MF, Wyatt JI, Tompkins DS, Taylor GR, Quirke P 1991: Direct polymerase chain reaction test for detection of *Helicobacter pylori* in humans and animals. J Clin Microbiol **29**: 2543-2549

Hooi JKY, Lai WY, NG WK, Suen MMY, Underwood FE, Tanyingoh D, Malfertheiner P, Graham DY, Wong VWS, Wu JCY, Chan FKL, Sung JJY, Kaplan GG, NG SC 2017: Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. Gastroenterology **153**: 420-429

International standard ISO TS 17728 2015: Microbiology of the food chain – Sampling techniques for microbiological analysis of food and feed samples. Geneva: European Committee for Standardization. 17 p.

Kotzekidou P 2016: Food hygiene and toxicology in ready-to-eat foods. San Diego: Academic Press, 474 p.

Khaji L, Banisharif G, Alavi I 2017: Genotyping of the *Helicobacter pylori* isolates of raw milk and traditional dairy products. Microbiol Res **8**: 7288

Liang T, Long H, Zhan Z, Zhu Y, Kuang P, Mo N, Wang Y, Cui S, Wu X 2022: Simultaneous detection of viable *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* in bird's nest, donkey-hide gelatine, and wolfberry using PMA with multiplex real-time quantitative PCR. Food Sci Nutr **17**: 3165-3174

Meng J, Doyle MP 1997: Emerging issues in microbiological food safety. Annu Rev Nutr **17**: 255-275

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

Mengistu DA, Tolera ST 2020: Prevalence of microorganisms of public health significance in ready-to-eat foods sold in developing countries: systematic review and meta-analysis. Int J Food Sci **2020**: 8867250

Mengistu DA, Belami DD, Tefera AA, Asefa AY 2022: Bacteriological quality and public health risk of ready-to-eat foods in developing countries: systematic review and meta analysis. Microbiol Insights **15**: 117863612211139

Mousavi S, Dehkordi FS, Rahimi E 2014: 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

Miyaji H, Azuma T, Ito S, Abe Y, Gejyo F, Hashimoto N, Sugimoto H, Suto H, Ito Y, Yamazaki Y, Kohli Y, Kuriyama M 2000: *Helicobacter pylori* infection occurs via close contact with infected individuals in early childhood. J Gastroenterol Hepatol **15**: 257-262

Ng CG, Loke MF, Goh KL, Vadivelu J, Ho B 2017: Biofilm formation enhances *Helicobacter pylori* survivability in vegetables. Food Microbiol **62**: 68-76

Oderda G 1999: Transmission of *Helicobacter pylori* infection. Can J Gastroenterol **13**: 595-597.

Poms RE, Tatini SR 2001: Survival of *Helicobacter pylori* in ready-to-eat foods at 4°C. Int J Food Microbiol **63**: 281-286

Quaglia NC, Dambrosio A 2018: *Helicobacter pylori*: A foodborne pathogen? World J Gastroenterol **24**: 3472-3487

Quaglia NC, Dambrosio A, Normanno G, Celano GV 2009: Evaluation of a Nested-PCR assay based on the phosphoglucosamine mutase gene (*glmM*) for the detection of *Helicobacter pylori* from raw milk. Food Control **20**: 119-123

Ranjbar R, Farsani FY, Dehkordi FS 2019: Antimicrobial resistance and genotyping of *vacA*, *cagA*, and *iceA* alleles of the *Helicobacter pylori* strains isolated from traditional dairy products. J Food Saf **39**:12594

Stevenson TH, Bauer N, Lucia LM, Acuff GR 2000: Attempts to isolate *Helicobacter* from cattle and survival of *Helicobacter pylori* in beef products. J Food Prot **63**: 174-178

Talaei R, Souod N, Momtaz H, Dabiri H 2015: Milk of livestock as a possible transmission route of *Helicobacter pylori* infection. Gastroenterol and Hepatol Bed Bench **8**: 30-36

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

Velázquez M, Feirtag JM 1999: *Helicobacter pylori*: Characteristics, pathogenicity, detection methods and mode of transmission implicating foods and water. Int J Food Microbiol **53**: 95-104

Zamani M, Vahedi A, Maghdouri Z, Shokri-Shirvani J 2017: Role of food in environmental transmission of *Helicobacter pylori.* Caspian J Intern Med **8**: 146-152