Milk and dairy products as a possible source of environmental transmission of *Helicobacter pylori*

Zora Šťástková, Pavlína Navrátilová, Aneta Gřondělová

University of Veterinary and Pharmaceutical Sciences Brno,
Department of Animal Origin Food & Gastronomic Sciences, Brno, Czech Republic

Abstract

Helicobacter pylori (*H. pylori*) is found worldwide, and it is estimated that more than 50% of the population is infected. The presence of *H. pylori* in the gastrointestinal tract is associated with the occurrence of serious human diseases such as chronic gastritis, duodenal ulcer or gastric cancer. Therefore, the International Agency for Research on Cancer classified *H. pylori* as a Group 1 carcinogen in 1994. The routes of *H. pylori* transmission are not yet precisely known. Many authors agree that humans may become infected by *H. pylori* through foodstuffs. Milk is one of the basic foods of people of all age categories. *Helicobacter pylori* has been isolated from milk derived from various livestock, such as cows, sheep, goat, camel or buffalo milk. This review research paper indicates that *H. pylori* may be present in different types of foods and suggests that consumption of milk and selected dairy products may be a source of *H. pylori* infection for humans.

In 1982, Australian pathologists Robin Warren and Barry Marshall first isolated *H. pylori* bacterium from gastric tissue. However, this bacterium was originally named *Campylobacter pyloridis,* which was later corrected to *Campylobacter pylori*, on the basis of similar properties with representatives of *Campylobacter* spp., such as the habitat and the culture medium for isolation. However, more detailed studies indicated that *C. pylori* should be included in a new genus. The term *Helicobacter pylori*, used since 1985, means a spiral rod in the lower part of the stomach (Gomes and De Martinis 2004).

It is a gram-negative, microaerophilic motile bacterium colonising the gastric mucosa. The presence of *H. pylori* in the gastrointestinal tract is associated with the occurrence of serious human diseases such as chronic gastritis, duodenal ulcer or gastric cancer. Therefore, the International Agency for Research on Cancer, established within the World Health Organization, classified *H. pylori* as a Group 1 carcinogen in 1994 (Guessoum et al. 2018).

Epidemiology

Helicobacter pylori bacterium is found worldwide, and it is estimated that more than 50% of the population is infected (Kao et al. 2016). Humans can become infected with this bacterium all throughout their lives (Quaglia and Dambrosio 2018). *Helicobacter pylori* has been reported to cause gastritis, peptic ulcer, adenocarcinoma, and MALT (mucosa-associated lymphoid tissue) lymphoma. According to the available data, 20 to 90% of hospitalised patients with gastrointestinal disorders were infected with *H. pylori* strains (Talimkhani and Mashak 2017). The prevalence of *H. pylori* infection depends to a large extent on age, geographical area or the socio-economic status (Brown 2000).

Address for correspondence:
MVDr. Zora Šťástková, Ph.D.
University of Veterinary and Pharmaceutical Sciences Brno
Department of Animal Origin Food & Gastronomic Sciences
Palackého tř. 1946/1, 612 42 Brno
Czech Republic

Phone: +420 541 562 721
E-mail: stastkovaz@vfu.cz
http://actavet.vfu.cz/

The socio-economic status includes a number of factors. The increased incidence of infection in the population is mainly due to high population density, low levels of hygiene or a lack of sanitation. Studies have shown that contracting the *H. pylori* infection is closely associated with a low socioeconomic status (Mitchell and Katelaris 2016).

There has been a significant difference in the prevalence of the *H. pylori* infection between developing and developed countries (Perez-Perez et al. 2004). *Helicobacter pylori* was detected in more than 80% of the population in developing countries (Quaglia and Dambrosio 2018). In these countries, people are more often exposed to sources of infection than people from developed countries (Vale and Vítor 2010). In developed countries, the prevalence of infection is lower, below 40%. While in developing countries, young people are also affected by the infection, in developed countries, the incidence is significantly lower in children than in adults (Quaglia and Dambrosio 2018). According to the available studies, the infection prevalence in the world has been decreasing due to improved sanitary conditions (Zamani et al. 2017).

The assessment of pathogenic virulence genes and genotypes is important for the epidemiology of *H. pylori*. VacA (vacuolating cytotoxin A) and *cagA* (cytotoxin associated gene A) are the most important markers of virulence detected in *H. pylori* strains. They are isolated in cases of gastrointestinal disorders but also from foodstuffs (Talimkhani and Mashak 2017). The gene encoding VacA belongs to a group of genes with a variable genotype or structure. It is associated with damage to gastric epithelial cells. This gene is found in virtually all *H. pylori* strains. It is polymorphic and includes variable signalling regions (type *s1* or type *s2*), a mid-region (type *m1* or type *m2*) and a recently identified intermediate-region (type *i1*, *i2* and *i3*) (Palframan et al. 2012; Saeidi and Sheikhshahrokh 2016). Type *s1* is further divided into subtypes *s1a*, *s1b*, and *s1c*; and *m1* into subtypes *m1a* and *m1b* (Gilani et al. 2017). The mosaic combination of allelic types with *s* and *m* regions determines a specific cytotoxin and subsequently the pathogenicity of the bacterium (Hemmatinezhad et al. 2016). For example, the combination of *s1/m1* alleles is associated with an increased risk of gastric cancer (Kao et al. 2016). *CagA* has been associated with ulceration, inflammation, and gastric cancer (Talimkhani and Mashak 2017).

Urease is another important virulence factor, in addition to the already mentioned VacA and *cagA*. Urease is an enzyme hydrolysing urea to carbon dioxide and ammonia, thus ensuring the survival of *H. pylori* in an acidic environment (Velázquez and Feirtag 1999). However, ammonia production damages the gastric epithelium and carcinogenic substances are formed in association with neutrophil metabolites, which may be involved in tumour development in this area (Roesler et al. 2014).

Flagella are an essential factor of virulence involved in the *H. pylori* colonisation process. The composition of the flagella is as follows: a membrane, filaments, lipopolysaccharides and proteins (Sukri et al. 2020). The *flaA* and *flaB* genes encode flagellin proteins, essential for perfect motility and persistent gastric mucosal infection (Cellini and Donelli 2000).

Helicobacter pylori virulence factors may also include adhesins located on the surface of a bacterial cell, by which the bacterium adheres to receptors in the gastric epithelium. These include BabA (blood-antigen binding protein A), SabA (sialic acid-binding adhesin), as well as NAP (neutrophil-activating protein), Hsp60 (heat shock protein 60), AlpA and AlpB (adherence-associated proteins), HopZ (outer membrane protein), and LabA (lacdiNAc-binding adhesin) (Kao et al. 2016).

Microbiological characteristics

Helicobacter pylori is a spiral-shaped Gram negative bacterium which measures 2 to 4 μm in length and ranges in width from 0.5 to 1 μm (Kusters et al. 2006). The bacterium may occur in three forms (Andersen and Rasmussen 2009):

- A viable spiral form which is culturable (infectious);
- A viable cocoid form, non-culturable (inability to grow on agar plates);
- A non-viable degenerative and non-culturable form.

The change in the *H. pylori* shape from a rod to a cocoid form occurs under adverse environmental conditions, such as insufficient nutrient supply (Andersen and Rasmussen 2009).

The bacteria require a microaerophilic environment. For optimal growth, the oxygen level should be 2 to 5% and the carbon dioxide level from 5 to 10%. The atmosphere should also have high air humidity (98%). The standard composition of the atmosphere for the growth of *H. pylori* used in laboratories is 5% O_2 + 10% CO_2 + 85% N_2 (Kusters et al. 2006). As specified by Jiang and Doyle (1998), the bacterium is able to grow even at pH 4.5. However, they recorded optimal growth at pH from 5.5 to 7.3. In their study, *H. pylori* also did not grow in an environment with 2% NaCl. However, in an environment of 0.5 to 1% NaCl, it grew relatively well. The growth of *H. pylori* also requires water activity in the range of 0.96 to 0.98, suggesting that the bacterium is unable to grow in many types of foodstuffs (Jiang and Doyle 1998).

The motility of the bacterium is ensured by four to six unipolar sheathed flagella with a spherical extension at the end. This part is an extension of the flagellar sheath. Each flagellum is approximately 30 μm long (Dunn et al. 1997). *Helicobacter pylori* grows from 30 to 37 °C, with the optimal growth at 37 °C. Growth at 42 °C is variable, while the bacterium is unable to grow at 25 °C (Velázquez and Feirtag 1999). In the case of culturing *H. pylori*, it is necessary to enrich the substrate with certain supplements, such as sheep or horse blood, haemoglobin, yolk emulsion, etc. (Quaglia and Dambrosio 2018). *Helicobacter pylori* shows a positive catalase and oxidase reaction, as well as strong urease positive activity. Bacteria show a negative reaction to hippurate (Velázquez and Feirtag 1999).

Transmission pathways of *H. pylori*

The routes of *H. pylori* transmission are not yet precisely known (Vale and Vítor 2010). The infectious dose of *H. pylori* is low and therefore even a small number of *H. pylori* cells may pose a potential health risk to humans (Quaglia et al. 2020). There has been a study aimed at determining the minimum infectious dose in humans, which has found that the minimum infectious dose is close to the value of 10^5 of colony forming units (CFU) (Graham et al. 2004). The available scientific data indicate that the spread of *H. pylori* has two possible alternatives, namely the transmission from human to human and through the environment (water, milk, etc.) to human (Zamani et al. 2017).

Person to person transmission may take place through the oral-oral route, the faecal-oral route, and the gastro-oral route. However, there are no data to determine the predominance of either of the transmission pathways (Quaglia et al. 2020). The gastro-oral transmission route may take place through gastric juices, gastroesophageal reflux, or gastric regurgitation (Brown 2000; Vale and Vítor 2010). This has been confirmed by a study conducted by Parsonnet et al. (1999), when *H. pylori* was present in large amounts in vomit. The vomiting process may spread the bacteria into the air. However, the aerosol is an unlikely source of infection due to the short duration of contamination. *Helicobacter pylori* DNA has also been detected in human faeces (Brown 2000). In the case of oral-oral transmission, studies confirm that saliva can serve as a *H. pylori* transmission vector. Some scientists have been successful in isolating *H. pylori* from dental plaque (Brown 2000).

Helicobacter pylori primarily colonizes the antral part of the human stomach. Some bacteria adhere to the gastric mucosa and some remain in the lumen of the stomach as non-adhering bacteria. These microorganisms can then continue freely into the intestine and,

together with the faeces, enter the environment. In the case of dyspepsia (e.g. vomiting), non-adherent bacteria may enter the oral cavity. Numerous studies have confirmed the presence of *H. pylori* DNA in the previously mentioned saliva and dental plaque (Andersen and Rasmussen 2009).

Animals, foodstuff, and their importance in the *H. pylori* transmission

Many authors agree that humans may become infected by *H. pylori* through foodstuffs (Fujimura et al. 2002; Angelidis et al. 2011; Mousavi et al. 2014). Some scientists refer to this bacterium as a pathogen causing foodborne illness. This is due to some microbiological and epidemiological properties of this microorganism. For example, there is a high prevalence of infection in the family or among individuals living in institutions (Quaglia et al. 2007).

The foodstuffs which could theoretically provide conditions suitable for the survival of *H. pylori* are those whose water activity is higher than 0.97 and the pH is in the range of 4.9–6.0. Therefore, the bacterium is likely to grow in most foods. Examples of foods in which *H. pylori* has been detected are provided in Table 1. The bacterium is characterised by its ability to survive for a long time in an environment with high humidity and low acidity, especially when stored in the cold (Gomes and De Martinis 2004). It is this capacity of *H. pylori* to survive in food that supports the theory that primary food contamination (animal reservoir) or secondary contamination as a result of improper handling (human reservoir) may serve as a means of *H. pylori* transmission (Vale and Vítor 2010).

Table 1. Detection of the occurrence of *H. pylori* in selected foodstuffs (adapted from Quaglia and Dambrosio 2018).

Foodstuff type	Method	Number of samples	Percentage of positive samples (%)
Raw sheep milk	Culture	63	2.60
	PCR		63.00
Raw cow's milk	Nested-PCR	110	50.00
Raw goat milk	Nested-PCR	160	25.60
Raw buffalo milk	PCR	80	15.00
Raw camel milk	PCR	60	13.30
Cheese (traditional Iranian)	PCR	100	30.00
Butter (traditional Iranian)	PCR	100	5.00
Cream (traditional Iranian)	PCR	100	15.00
Ice cream (traditional Iranian)	PCR	100	27.00
Raw meat	PCR	400	26.25
Hamburger	Nested-PCR	80	1.42
Lettuce	PCR	80	13.75
Spinach	PCR	80	6.25

Helicobacter pylori has been detected in drinking and sea water, as well as in foods of animal origin, and therefore animals are believed to serve as a reservoir for the microorganism (Quaglia et al. 2008). This has been confirmed by several studies which have found that there is a high prevalence of antibodies to *H. pylori* in slaughterhouse workers, veterinarians or butchers (Tabatabaei 2012). Even domestic animals can be a source of *H. pylori*. This bacterium was detected in dogs and more rarely in cats (Neiger and Simpson 2000; Erginsoy at al. 2006). Furthermore, *H. pylori* has also been detected in raw vegetables. Several studies have been conducted showing that vegetable contamination may occur through irrigation water (Zamani et al. 2017). According to

studies, *H. pylori* DNA has been detected inside *Candida* yeasts, which can serve as a reservoir or method for protecting the bacterium in the natural environment. These yeasts are often found in water, in food, but also in the human gastrointestinal or urinary tract (Vale and Vítor 2010; Zamani et al. 2017).

Milk and dairy products

Milk is one of the basic foods of people of all age categories. It is usually processed into dairy products such as butter, yogurt, cream or cheese. Millions of people around the world consume milk and dairy products on a daily basis, and therefore the hygienic quality of milk is very important (Mousavi et al. 2014).

Milk contamination usually occurs during milking. *Helicobacter pylori* enters the surface of the mammary gland (udder) from various sources, contaminated with the faeces of infected animals. The source may include soil, manure, bedding, or water. Poor hygiene practices on farms contribute to the spread of bacteria from animal to animal during milking, through contaminated milking equipment or the hands of milkers or the wipes used (Osman et al. 2015). Milk contaminated during collection is deemed to be one of the most likely carriers of infection (Quaglia et al. 2007).

Helicobacter pylori has been isolated from milk derived from various livestock, such as cow's, sheep, goat, camel or buffalo milk (Zamani et al. 2017). The frequency of *H. pylori* occurrence in the milk of various livestock is provided in Table 2.

Table 2. Frequency of occurrence of *H. pylori* in milk originating from various livestock (Verraes et al. 2014).

Raw milk type	Occurrence frequency (%)
Cow's milk	From 14.1 to 50.0
Goat milk	From 8.7 to 25.6
Sheep milk	0 to 60.3
Buffalo milk	2.45
Camel milk	6.0

Based on this fact, livestock may be a potential source of *H. pylori* and a potential risk both for people handling these animals and for consumers of their products (Guessoum et al. 2018). The ability of *H. pylori* to survive in an acidic environment is dependent on urea. It is present in milk and may be responsible for the long-term survival of the bacterium in this food (Jiang and Doyle 1998).

According to a study conducted by Fujimura et al. (2002) in Japan, semi-nested PCR was used to detect *H. pylori* DNA in 72% of raw cow's milk samples. In Greece, Angelidis et al. (2011) detected *H. pylori* in 20% of raw cow's milk samples. Quaglia et al. (2008) found H. *pylori* in raw sheep, cow's and goat milk originating in northern Italy. The prevalence of this bacterium was 33.0%, 50.0%, and 25.6%. The team of authors Ranjbar et al. (2018) studied traditional dairy products originating in Iran. Specifically, they tested cheese, cream, butter and yogurt for the presence of *H. pylori*. They showed that 11.53% of the cheese samples, 5.00% of the cream samples, 4.00% of the butter samples and 1.11% of the yoghurt samples were positive for *H. pylori*. The detection of this pathogen in dairy products made in Iran has also been addressed by Mousavi et al. (2014). In their work, they examined dairy products made from raw milk, namely cheese, cream, butter and ice cream. *Helicobacter pylori* was detected in 30% of cheese samples, 15% of cream samples, 5% of butter samples, and 27% of ice cream samples. The high incidence of *H. pylori* in traditional Iranian cheeses may be due in particular to the low temperature used to treat raw milk, around 40 °C. In yogurt, unlike milk, *H. pylori* is unable to survive. The growth of *H. pylori* is affected by the acidic pH and organic acids produced by the growth of lactic acid bacteria (Quaglia and Dambrosio 2018).

In some studies, researchers failed to isolate *H. pylori* from milk. The team of authors Jiang and Doyle (2002) were unable to detect *H. pylori* in raw cow's milk using the PCR method. *Helicobacter pylori* was not isolated from raw sheep milk originating in Turkey using the culture method applied by Turutoglu and Mudul (2002). Tabatabaei (2012)

failed to detect the presence of *Helicobacter* spp. in a single milk sample using a culture method from 20 raw cow's milk samples from south-western Iran. This could be due to the presence of *H. pylori* in a cocoid, viable but non-culturable form (VNC, viable non-culturable form). This is because this form cannot be detected using the culture method. As reported by Bianchini et al. (2015), the impossibility to isolate *H. pylori* using the culture method may be due to an overgrowth of contaminating microflora, making it impossible to identify small *H. pylori* colonies after the culturing. Another issue may consist in the selective media for culturing *H. pylori* which have been primarily developed for culturing microorganisms from clinical samples whose microflora is different from the food microflora. The impossibility to isolate *H. pylori* from samples may be due to the geographical distribution of the bacterium (Jiang and Doyle 2002).

Several studies have been conducted addressing the survival of *H. pylori* in milk and dairy products. According to the study carried out by Fan et al. (1998), *H. pylori* was found to be able to survive in artificially contaminated pasteurised milk for the period of 10 days at a storage temperature of 4 °C. According to the research conducted by Quaglia et al. (2007), *H. pylori* was able to survive in artificially contaminated UHT milk for a period of 12 days, and in pasteurized milk for a period of 9 days at a storage temperature of 4 °C.

Probiotics and *H. pylori*

Today, antibiotics (amoxicillin, clarythromycin, metronidazole, etc.) are used in combination with antisecretory therapy to treat infections caused by *H. pylori*. However, there is a problem consisting in the increasing antimicrobial resistance of *H. pylori* to antibiotics. Therefore, research in this area focuses on new therapeutic solutions, such as the use of probiotics in the treatment of *H. pylori* infection (Švestka 2011).

Probiotics are defined as living, non-pathogenic organisms added to feed or food and intended to have a positive effect on the consumer by improving the balance of their intestinal microflora (Cazzato et al. 2004). In clinical practice, the most commonly used probiotics include *Lactobacillus* spp., *Bifidobacterium* spp., *Bacillus* spp., *Escherichia coli*, *Streptococcus thermophilus*, and *Sacharomyces boulardii* (Franceschi et al. 2007).

Effect of probiotics on *H. pylori*

Studies indicate that probiotics have various effects. First, there may be in direct competition with *H. pylori*, causing the inhibition of adherence and the production of metabolites and antimicrobial molecules. Second, probiotics reduce the incidence of side effects associated with antibiotics administration (Cazzato et al. 2004).

In a healthy person, lactobacilli are the dominant bacteria in the stomach. Their concentration here ranges from 0 to 10^3 CFU/ml of liquid. The abilities of lactobacilli of human origin are assumed to include the colonisation of the epithelial mucosa surface. They may thus interfere with pathogens *in vivo* (Cazzato et al. 2004). Owing to the various components of the bacterial surface, *H. pylori* may tightly adhere to epithelial cells. A study was conducted in which two of the nine *Lactobacillus reuteri* (*L. reuteri*) strains were able to bind to glycolipid receptors (asialo-GMI and sulphatide) and thus inhibit the binding of *H. pylori* to these receptors. With the help of these strains of *L. reuteri*, *H. pylori* infection could be prevented in the initial stage of colonisation of the gastric mucosa (Felley and Michetti 2003). Some lactobacilli have the capacity to synthesize antimicrobial compounds belonging to bacteriocins. Other substances secreted by these bacteria include lactic acid, acetic acid and hydrogen peroxide. Some authors consider the relatively large amount of lactate produced by lactobacilli to be an inhibitory factor in *H. pylori*. Lactic acid, which has an antimicrobial effect by lowering the pH, can inhibit *H. pylori* urease (Lesbros-Pantoflickova et al. 2007). The strains capable of inhibiting

or killing *H. pylori* include *L. acidophilus*, *L. casei*, *L. johnsonii* La1, and *L. salivarius* (Hamilton-Miller 2003). However, there are specific probiotic strains which show antibacterial activity due to the production of antibiotic compounds. An example may include the supernatant of *Bacillus subtilis*. The inhibition of *H. pylori* growth is thus independent of organic acid concentration or pH (Cazzato et al. 2004).

There may be side effects (e.g. bloating, nausea, diarrhoea, constipation, vomiting, loss of appetite, or epigastric pain) occurring when taking antibiotics (to treat the *H. pylori* infection) at high doses. The side effects may reduce the effectiveness of the therapy and affect the patient's treatment cooperation. Interrupting the therapy due to the occurrence of side effects may increase the risk of developing antibiotic-resistant strains. Therefore, new strategies have been developed to improve the tolerability of *H. pylori* eradication therapies, including the use of probiotics, among other things. This has been confirmed by a study in previously asymptomatic patients infected with *H. pylori*, undergoing standard triple eradication therapy and receiving *Bacillus clausii* supplementation for two weeks. The decreased occurrence of nausea, diarrhoea and epigastric pain has been reported in these patients. Similar results were obtained when using *S. boulardii* and *L. casei*. In another study, randomly selected patients (infected by *H. pylori*) were divided into two groups, one taking probiotics (*Lactobacillus* GG or *S. boulardii* or a combination of *Lactobacillus* spp. and bifidobacteria), while the other receiving placebo during the therapy and seven days after that. A significantly lower incidence of diarrhoea and taste disturbances was observed in patients taking probiotics (Cazzato et al. 2004; Franceschi et al. 2007).

Conclusion

Helicobacter pylori is a bacterium widely spread in the human population. However, the routes of transmission to humans have not been elucidated. It has been found that this bacterium is present in many types of food (vegetables, meat, milk, dairy products, etc.). This review focuses on the occurrence of *H. pylori* in milk and dairy products, which are among the basic foods. The studies indicate that *H. pylori* may be present in these foods, suggesting that consumption of milk and selected dairy products may be a source of *H. pylori* infection for humans. In order to confirm the unambiguous transmission of bacteria by food or water to humans, more research is needed into other foodstuffs of animal origin.

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