

Helicobacter pylori: A foodborne pathogen?

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Abstract

Helicobacter pylori (*H. pylori*) is an organism that is

widespread in the human population and is sometimes responsible for some of the most common chronic clinical disorders of the upper gastrointestinal tract in humans, such as chronic-active gastritis, duodenal and gastric ulcer disease, low-grade B-cell mucosa associated lymphoid tissue lymphoma of the stomach, and gastric adenocarcinoma, which is the third leading cause of cancer death worldwide. The routes of infection have not yet been firmly established, and different routes of transmission have been suggested, although the most commonly accepted hypothesis is that infection takes place through the faecal-oral route and that contaminated water and foods might play an important role in transmission of the microorganism to humans. Furthermore, several authors have considered *H. pylori* to be a foodborne pathogen because of some of its microbiological and epidemiological characteristics. *H. pylori* has been detected in drinking water, seawater, vegetables and foods of animal origin. *H. pylori* survives in complex foodstuffs such as milk, vegetables and ready-to-eat foods. This review article presents an overview of the present knowledge on the microbiological aspects in terms of phenotypic characteristics and growth requirements of *H. pylori*, focusing on the potential role that foodstuffs and water may play in the transmission of the pathogen to humans and the methods successfully used for the detection of this microorganism in foodstuffs and water.

Key words: *Helicobacter pylori*; Viable but nonculturable state; Foodborne pathogen; Food; Water; Animal reservoirs; Culture methods; Molecular methods

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Core tip: To date, the transmission routes and reservoirs of *Helicobacter pylori* (*H. pylori*) are topics of debate. Epidemiological evidence and the occurrence of *H. pylori* in foods of animal origin, vegetables and water corroborate the hypothesis advanced by numerous authors that *H. pylori* may be a foodborne pathogen. The present review is focused on the

evidence supporting the role of foods and water in the transmission of *H. pylori* to humans and on the methods for detecting the pathogen in foodstuffs and water.

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INTRODUCTION

The first isolation of *Helicobacter pylori* (*H. pylori*) in 1982 by Marshall and Warren^[1,2] marked a turning point in understanding gastrointestinal microbial ecology and disease^[3]. Following the initial scepticism regarding the aetiologic importance of this organism, it is now recognized that infections with *H. pylori* are linked to some of the most common chronic clinical disorders of the upper gastrointestinal tract in humans^[4]. In fact, *H. pylori* has been acknowledged as a major cause of chronic-active gastritis and is associated with duodenal and gastric ulcer disease, low-grade B-cell mucosa-associated lymphoid tissue lymphoma of the stomach (MALToma)^[5], and gastric adenocarcinoma, which is the third leading cause of cancer death worldwide^[4,6,7]. Furthermore, *H. pylori* has been linked to a variety of extra-gastric disorders, including coronary heart disease, dermatological disorders such as rosacea and idiopathic urticaria, autoimmune thyroid disease, thrombocytopenic purpura, and iron deficiency anaemia^[8].

Human infection by *H. pylori* is a great public health hazard because *H. pylori* colonizes the gastric mucosa of approximately half of the world's population^[9-12]. The infection is usually acquired in infancy and early childhood, and it is long lasting, often remaining for the entire lifespan^[13]. The prevalence of *H. pylori* shows large geographical variation, with infection rates much higher in developing countries (in some areas > 85%) than in Europe and North America (approximately 30%-40%)^[14,15]. In various developing countries, more than 80% of the population is *H. pylori* positive, even at young ages^[16]. The prevalence of *H. pylori* in industrialized countries generally remains under 40% and is considerably lower in children and adolescents than in adults and elderly people. A comparison of prevalence rates by age suggests that the acquisition of *H. pylori* is decreasing in recent cohorts, and this finding is most apparent in developed countries and may be linked to improvements in hygiene practices. Furthermore, it has been estimated that between two and 20 percent of people infected with *H. pylori* develop peptic ulcer disease^[17].

Although *H. pylori* can cause severe illnesses with a high rate of morbidity and mortality, the complex interactions between this microbe and humans,

particularly its transmission pathways to humans and reservoirs, are largely unknown, although multiple routes of transmission have been suggested^[3,18-20]. The current literature suggests that the transmission of *H. pylori* occurs from person to person *via* the oral-oral, faecal-oral, and gastric-oral routes and that the infection dose for humans is low^[6,21]. *H. pylori* may be a sex-transmitted pathogen^[22,23] and may lead to fibrocystic breast changes^[24]. The oral cavity can be primarily colonized by *H. pylori*, and this can be linked to later gastric infection^[25]. Faecal-oral transmission has more important implications than oral-oral transmission because *H. pylori* may occur in food and water supplies subsequent to faecal contamination^[26]. Furthermore, several authors have considered *H. pylori* to be a foodborne pathogen because of its microbiological and epidemiological characteristics^[6,10,27-31]. Information on the distribution of *H. pylori* in water, vegetables and foods of animal origin is critical in determining its potential transmission in foods.

This review article presents a brief overview of the present knowledge on the microbiological characteristics of *H. pylori* in terms of its phenotypic characteristics and growth requirements, focusing on the potential role that foodstuffs and water may play in the transmission of this pathogen to humans and the methods for isolating and detecting this microorganism in foodstuffs and water.

LITERATURE SEARCH

A PubMed search was conducted using the following keywords and phrases: "*Helicobacter pylori*, *Helicobacter pylori* and food, *Helicobacter pylori* and milk, *Helicobacter pylori* and water, VBNC, survival of *Helicobacter pylori*". In addition, we performed a manual review of the reference lists of the primary and review articles to ensure identification of all relevant articles.

MICROBIOLOGICAL CHARACTERISTICS

Phenotypic characteristics

H. pylori was originally thought to be a species belonging to the genus *Campylobacter* and was first named *Campylobacter pyloridis*, which was later corrected to *Campylobacter pylori* (*C. pylori*)^[32]. Because subsequent 16S rRNA sequence analysis showed that the distance between the species belonging to the genus *Campylobacter* and *C. pylori* was sufficient to exclude *C. pylori* from this genus^[33], it was renamed *Helicobacter pylori*^[34], the first member of the new genus *Helicobacter*.

H. pylori organisms are spiral or curved bacilli ranging from 0.3 to 1.0 μm in width and 1.5 to 10.0 μm in length; they are gram-negative and assume a rod-like shape when cultured on solid medium^[34]. Furthermore, after prolonged *in vitro* culture and under adverse environmental conditions, such as an insufficient supply of nutrients, desiccation, lack of protection against oxygen, and exposure to antimicrobial agents, *H. pylori* can survive entering the viable but non-culturable

(VBNC) state, changing its rod-like shape to a coccoid shape^[35-37].

When this morphological change occurs, *H. pylori* is unable to grow on agar plates using conventional cultivation methods^[38-40].

Bacteria in the VBNC state maintain their metabolic activity, pathogenicity and ability to return to active regrowth conditions^[41,42]. For *H. pylori*, the ability to return to active regrowth conditions has not yet been proven. Nevertheless, the aptitude of *H. pylori* to overcome stressed conditions is very significant for public health^[29], even if the role of VBNC in the transmission of *H. pylori*, especially by food and water, is still controversial.

H. pylori is motile and usually possesses four to six unipolar-sheathed flagella, which may be an adaptation to survive in gastric juices^[3,43].

Growth requirements

Since the discovery of *H. pylori*, bacterial culture has been used as a routine diagnostic test and is considered the gold standard. *H. pylori* culture is recommended for performing antibiotic susceptibility testing if primary resistance to clarithromycin is higher than 20% or after failure of second-line treatment^[44]. Despite the long use of bacterial culture, to date, there are no defined media for the selective culture of *H. pylori* because of its fastidious nature with particular growth requirements of atmosphere, nutrient-rich media, high humidity (98%), and long incubation time (5-7 d)^[3,44].

H. pylori is a capnophilic organism that requires an atmosphere with a high level of CO₂ (from 5% to 10%). It has been considered a microaerophile, but the concentration of O₂ required for its growth is still a topic of discussion^[44].

H. pylori requires a complex culture substrate (solid or liquid) with some forms of supplementation, such as whole sheep or horse blood, haemoglobin, serum, coal, yeast, or yolk emulsion^[45,46], which may serve as nutritional substrates. These supplements also detoxify the medium and protect the microorganism^[7].

Furthermore, if isolation is attempted from samples with basic microbial flora, it is necessary to make the media selective through supplementation of several antibiotics^[47].

Growth in liquid media is enhanced by agitation, which allows gas dispersion and incubation in a CO₂-rich atmosphere^[31,47].

H. pylori grows within a temperature range of 30 °C to 37 °C, with optimum growth at 37 °C, but is not able to grow at 25 °C^[43]. At 42 °C, growth is variable^[29].

Similar to *C. jejuni*, *H. pylori* survives longer at 4 °C than at room temperature, and it grows within a pH range of 4.5 to 7.3, with optimum at pH 5.5. *H. pylori* grows well in the presence of 0.5% and 1% NaCl but not of 2% NaCl. The minimum water activity (a_w) for growth is between 0.96 and 0.98. These data suggest that this microorganism is most likely not able to grow in many types of food^[47].

H. pylori is catalase and oxidase positive; it is also

characterized by strong urease activity and is negative for hippurate and nitrate reduction, characteristics that discriminate it from species belonging to the genus *Campylobacter*^[29].

EVIDENCE SUPPORTING THE ROLE OF FOODS IN TRANSMISSION OF *H. PYLORI* TO HUMANS

Since 1997, when the transmission of *H. pylori* through water and foods was hypothesized for the first time, several studies have evaluated the survival and the presence of this microorganism in different foodstuffs (Tables 1 and 2).

One of the most important topics supporting this thesis was the phylogenetic proximity of *H. pylori* to *C. jejuni*, which led to the hypothesis that the transmission pathways described for the latter could also be applied to *H. pylori*^[27]. Several investigators have also considered *H. pylori* a foodborne pathogen based on some of its epidemiological characteristics^[27,28,30] such as the high prevalence of infection within closed family groups and among individuals living in institutions^[48]. These aspects suggest that in addition to direct transmission, this bacterium may be transmitted indirectly through a common source, such as through consumption of the "same foods at the same table"^[26]. The finding that the prevalence of *H. pylori* infection is greater in geographical areas in which the hygienic conditions of life are poor also supports this hypothesis^[30,49].

Additional indirect evidence of the transmission of *H. pylori* to humans through foods of animal origin has been provided by epidemiological studies on the presence of antibodies in slaughterhouse workers and in veterinary workers. The incidence rates in these workers were positive and were greater than those in workers who had no direct contact with carcasses^[50,51] (Figure 1).

Foods presenting intrinsic factors, such as a_w higher than 0.97 and pH ranging from 4.9 to 6.0, could theoretically provide conditions for *H. pylori* survival^[30,52].

Therefore, data on survival ability may be more important than concerns about the growth of the microorganism in foods when determining the role of foods in *H. pylori* transmission to humans^[53].

Survival of *H. pylori* in foodstuffs

Several studies have demonstrated the survival of *H. pylori* in water, milk, ready-to-eat foods, vegetables, pasteurized apple and orange juices, ground beef and dry fermented sausages^[26,53-60] (Table 1).

H. pylori is able to survive in artificially contaminated milk stored at 4 °C for several days (from 5 to 9 d in pasteurized milk and from approximately 6 to 12 d in sterile milk)^[26,53,54,56]. These findings corroborate the hypothesis that post-processed contaminated milk may play a more effective role than other foods in the transmission of *H. pylori* infection due to the intrinsic

Table 1 Studies evaluating the survival of *Helicobacter pylori* in artificially contaminated foods

Year	Food	Method	Observations	Ref.
1998	Sterilized milk	Bacterial count on chocolate agar	10 d at 4 °C 3 d at 25 °C	Fan <i>et al.</i> ^[54]
2001	Pasteurized milk water tofu, tofu, yogurt, lettuce and chicken	Bacterial count on tryptic soy agar, non-selective Wilkins-Chalgren Anaerobe blood agar and selective Wilkins-Chalgren Anaerobe blood agar	from 5 to 7 d in pasteurized milk, tofu and water tofu at 4 °C for up to 2 d in lettuce and raw chicken at 4 °C for up to 1 d in yogurt at 4 °C	Poms <i>et al.</i> ^[26]
2000	Ground beef packaged in vacuum and air	Bacterial count on <i>H. pylori</i> special peptone agar	6 d in ground beef packaged in air at 4 °C 3-6 d in ground beef packaged in vacuum at 4 °C 3 d in ground beef packaged in air and in vacuum at -18 °C	Stevenson <i>et al.</i> ^[55]
2002	Ground beef, sterile milk, and apple and orange juices	Bacterial count on brain heart infusion agar and horse serum	7 d in ground beef at 4 °C 11 d in irradiated ground beef at 4 °C 6 d in sterile milk at 4 °C 1 d in apple and orange juice at 4 °C and 25 °C	Jiang <i>et al.</i> ^[56]
2004	Lettuce and carrots	Bacterial count on <i>Helicobacter</i> special peptone agar and Columbia blood agar	3 d in lettuce at 8 °C 5 d in sterilized carrot at 8 °C 3 d in sanitized carrot at 8 °C	Gomes <i>et al.</i> ^[57]
2007	Sterile milk and pasteurized milk	Bacterial count on Wilkins-Chalgren anaerobe agar	12 d in sterile milk at 4 °C 9 d in pasteurized milk at 4 °C	Quaglia <i>et al.</i> ^[53]
2010	Spinach	Bacterial count on brucella blood agar, Wilkins-Chalgren anaerobe blood agar	6 d at 8 °C	Buck <i>et al.</i> ^[58]
2011	Traditional Turkish fermented sausage (<i>sucuk</i>)	Bacterial count on Wilkins-Chalgren anaerobe blood agar	7 d	Guner <i>et al.</i> ^[59]
2017	Spring onion, cabbage, lettuce and spinach	Bacterial count on non-selective Blood base agar with 5% horse blood	3 d in spring onion, lettuce and spinach 4 d in cabbage stored at 4 °C	Ng <i>et al.</i> ^[60]

characteristics of this organism^[53]. It is well known that *H. pylori*'s ability to survive in an acidic pH environment is urea dependent^[3], and because urea is present in milk^[61], the urea-dependent acid resistance of *H. pylori* may account for the long-term survival of *H. pylori* in this foodstuff^[47]. Moreover, the microorganism is able to survive in milk for longer than the best-before date on an open milk package, making milk a possible source of transmission of this microorganism to humans. In fact, although the *H. pylori* load contaminating milk under natural conditions is unknown (although it is presumably lower than that used *in vitro*), the infection dose for humans is low; thus, even a small number of *H. pylori* cells surviving in foods may represent a potential health hazard for consumers^[53].

Other studies have been conducted on the survival of microorganisms in other more complex foodstuffs. *H. pylori* survives for approximately 7 d in ground beef at 4 °C, up to 3 d at -18 °C^[55,56] and for only 2 d in prepacked boneless, skinless chicken thighs^[26]. Vacuum packaging has no impact on survival time. However, if the high level of background bacteria present in the ground beef is eliminated, survival time increases to an undetectable level (< 10 cfu/g) within 11 d^[55]. The fate of *H. pylori* during the fermentation process of a traditional Turkish fermented sausage (*sucuk*) was investigated. The results of this study showed that the microorganism could survive and grow during the

fermentation process of *sucuk* (22 °C for 7 d). A possible explanation is that some fermentation products, such as protein degradation compounds and CO₂, might have been used by this pathogen and that indigenous bacteria might have created a microenvironment suitable for *H. pylori* growth^[59]. In contrast, *H. pylori* is not able to survive in yogurt^[26] or pasteurized fruit juice^[56] because its growth is hampered by the acidic pH and organic acids from lactic acid bacteria growth^[62,63].

Survival time in vegetables is shorter: 3 d in sanitized lettuce and carrot stored at 8 °C, 4 d in sterilized carrot and 5 d in carrot packaged in a modified atmosphere^[57]. A possible explanation could be the not-robust nature of this bacterium that on the surface of vegetables is exposed to oxygen and desiccation as opposed to what happens in liquid food and the presence of a high load of natural bacterial flora. Moreover, *H. pylori* is able to survive in contaminated vegetables despite the abovementioned adverse conditions, as it is able to form biofilms^[60]. However, a study on the survival of *H. pylori* in artificially contaminated spinach showed that this bacterium is able to survive for up to 6 d in VBNC forms that are still viable and can maintain its virulence factors despite its lack of cultivability^[58].

Occurrence of *H. pylori* in foodstuffs

Based on these findings, several studies have attempted to prove the occurrence of *H. pylori* in foodstuffs (Table

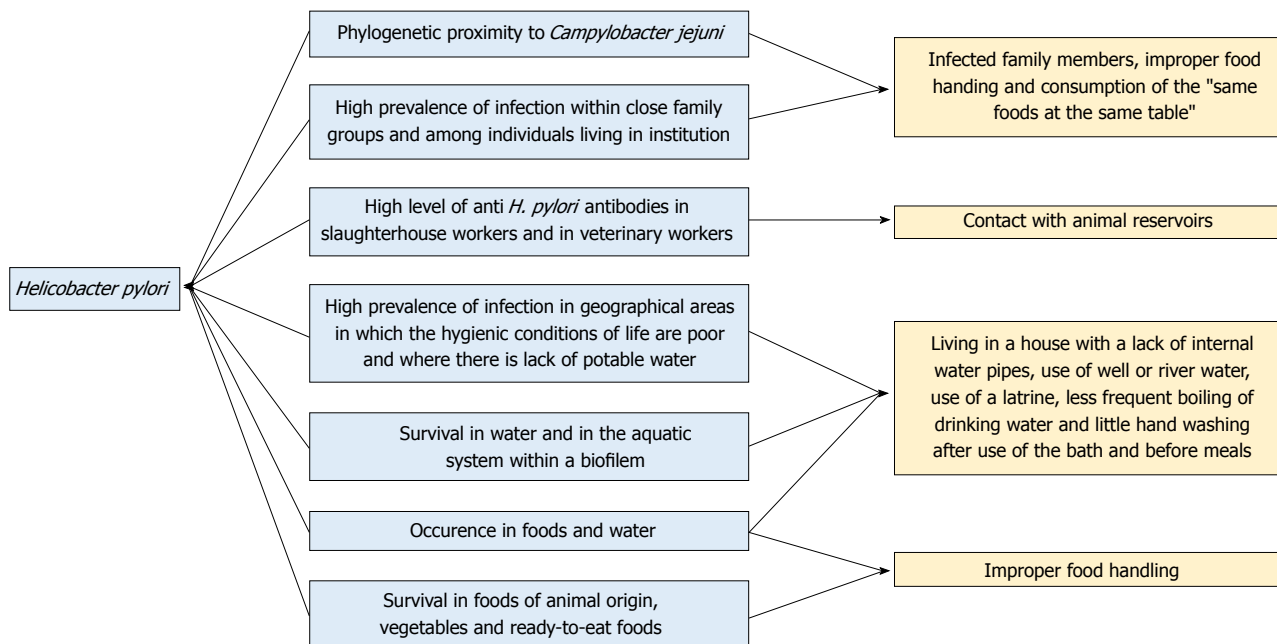


Figure 1 Evidence supporting the role of foods and water in the transmission of *Helicobacter pylori* to humans. In light blue is the epidemiological evidence supporting the hypothesis that *H. pylori* is a foodborne and/or a waterborne pathogen; in orange are the risks factors for *H. pylori* infection. *H. pylori*: *Helicobacter pylori*.

2). To the best of our knowledge, the first report about the presence of *H. pylori* in sheep milk was prompted by the observation that Sardinian shepherds with direct animal contact had a higher prevalence of infection than did their same-household siblings^[64]. *H. pylori* was isolated in 1 out of 38 PCR-positive raw sheep milk samples and in one out of 6 PCR-positive sheep gastric tissue samples^[65].

After these findings, *H. pylori* has rarely been isolated from raw milk samples^[66-69]. Bacteriological isolation of *H. pylori* occurred in one sample of raw cow milk out of 13 PCR-positive samples during a survey conducted in Japan^[66]. It was also isolated in 4 samples of raw cow milk out of 20 samples analysed in Greece^[67] and in Iran in 2 samples of raw sheep milk out of 11 PCR-positive samples and in 1 raw buffalo milk out of 15 PCR-positive samples^[68]. Afterward, Mousavi *et al*^[69] and Saedi *et al*^[70] reported a higher prevalence of *H. pylori* in raw cow, sheep, goat, buffalo and camel milks in Iran than that previously mentioned.

Furthermore, only a few studies have been carried out on the occurrence of *H. pylori* in dairy products other than milk. For example, in the survey of Mousavi *et al*^[69], 30% of Iranian traditional cheese, 15% of cream, 5% of butter and 27% of ice cream samples all made from unpasteurized milk were positive for *H. pylori*.

Compared to the few bacteriological isolations, the prevalence of *H. pylori* DNA is higher depending on the sensitivity of the method employed and the target gene^[65,66,68,71-73]. Conversely, these findings were not confirmed in the studies conducted by Jiang and Doyle^[56], Turutoglu *et al*^[74] and Bianchini *et al*^[75], which

failed to detect *H. pylori* in cow and sheep raw milk in the United States, Turkey and Italy, respectively, by PCR and bacteriological analysis.

The attempts to culture *H. pylori* from the majority of PCR-positive samples may have been unsuccessful for several reasons: the low number of contaminating bacteria in milk samples, the presence of VBNC forms that are not detectable by conventional microbiological culture-based protocols, and the relatively long period of storage before analysis, which could have affected the vitality of the few *H. pylori* cells present in the contaminated samples^[37,67,71].

Studies on the detection of *H. pylori* in food products other than milk are quite rare. *H. pylori* was isolated in 25%, 37%, 22%, 28% and 14% of cow, sheep, goat, buffalo and camel meat samples^[70] and in 1.42% and 12.5% of hamburger and minced beef samples, respectively^[76]. *H. pylori* DNA was detected in 36% and 44% of raw chicken and ready-to-eat raw tuna meat samples, respectively^[77]. Furthermore, Hemmatinezhad *et al*^[78] analysed 550 samples of ready-to-eat foods, detecting *H. pylori* in 74% of samples; olive salad (36%), restaurant salad (30%), fruit salad (28%) and soup (22%) were the most commonly contaminated. Additionally, Ghorbani *et al*^[79] recovered *H. pylori* in 60 out of 300 ready-to-eat food samples (20%), including ready-to-eat fish (15%), ham (8.33%), chicken sandwiches (5%), vegetable sandwiches (18%), meat sandwiches (10%), and minced meat (32%).

Few reports have addressed the occurrence of *H. pylori* in vegetables. In two surveys conducted in Iran, many of the vegetables analysed were positive for the presence of this microorganism: 13.72%^[80]

Table 2 Studies evaluating the occurrence of *Helicobacter pylori* in foods

Year	Food	Method	Number of samples <i>n</i>	Observations	Ref.
2001	Raw sheep milk	Culture and PCR	63 raw sheep milk	60% PCR positive samples 2.6% culture positive samples	Dore <i>et al</i> ^[65]
2002	Raw and pasteurized cow milk	Semi nested PCR, culture method and electron microscopy	18 raw cow milk 20 pasteurized milk	Raw milk: 72.2% semi-nested PCR positive samples; 1 culture positive sample Pasteurized milk: 55% semi-nested PCR positive samples	Fujimura <i>et al</i> ^[66]
2002	Raw sheep milk	Culture	440 raw sheep milk	0% positive samples	Turutoglu <i>et al</i> ^[74]
2008	Raw goat, sheep, and cow milks	Nested-PCR	160 raw goat milk 130 raw sheep milk 110 raw cow milk	25.6% positive goat milk 33% positive sheep milk 50% positive cow milk	Quaglia <i>et al</i> ^[71]
2008	Raw chicken and ready-to-eat raw tuna	Multiplex PCR	11 raw chicken 18 ready-to-eat raw tuna	36% positive raw chicken 44% positive ready-to-eat raw tuna	Meng <i>et al</i> ^[77]
2011	Raw cow milk	FISH	20	20% positive samples	Angelidis <i>et al</i> ^[67]
2012	Raw cow, sheep, goat, buffalo and camel milks	PCR	75 raw cow milk 58 raw sheep milk 42 raw goat milk 20 raw buffalo milk 15 raw camel milk	16.00% positive cow milk 13.79% positive sheep milk 4.76% positive goat milk 13.33% positive camel milk 20.00% positive buffalo milk	Rahimi <i>et al</i> ^[68]
2014	Milk and traditional dairy products	Culture and PCR	120 raw cow milk 100 raw goat milk 100 raw sheep milk 80 raw buffalo milk 60 raw camel milk 60 raw donkey milk 100 cheese 100 butter 100 cream 100 ice cream	16.6% positive cow milk 28% positive goat milk 35% positive sheep milk 15% positive buffalo milk 13.3% positive camel milk 0% positive donkey milk 30% positive cheese 15% positive cream 5% positive butter 27% positive ice cream	Mousavi <i>et al</i> ^[69]
2014	Vegetables and salad	Culture and PCR	60 salad 40 basil 40 radish 40 leek 80 spinach 80 lettuce 120 parsley	16.6% positive salad 12.5% positive basil 7.5% positive radish 20% positive leek 6.25% positive spinach 13.75% positive lettuce 6.6% positive parsley	Atapoor <i>et al</i> ^[81]
2014	Washed and unwashed vegetables	Culture and PCR	430 washed and unwashed vegetable	13.72% positive vegetables and salads	Yahaghi <i>et al</i> ^[80]
2015	Raw cow, sheep, goat, buffalo and camel milks	PCR	75 raw cow milk 58 raw sheep milk 42 raw goat milk 20 raw buffalo milk 15 raw camel milk	16.00% positive cow milk 13.79% positive sheep milk 4.76% positive goat milk 13.33% positive camel milk 20.00% positive buffalo milk	Talaei <i>et al</i> ^[72]
2015	Raw cow milk	Culture and nested PCR	50 raw cow milk	22% positive cow milk	Osman <i>et al</i> ^[73]
2015	Raw cow milk	Culture and nested PCR	163 raw cow milk	0% positive cow milk	Bianchini <i>et al</i> ^[75]
2016	Raw cow, sheep, goat, buffalo and camel milks and meats	Culture and PCR	420 raw milk 400 raw meat	21.90% positive raw milk 26.25% positive meat	Saedi <i>et al</i> ^[70]
2016	Ready-to-eat food	Culture and PCR	550 ready-to-eat food	13.45% positive ready-to-eat food	Hemmatinezhad <i>et al</i> ^[78]
2016	Ready-to-eat food and minced meat	Culture and PCR	60 ready-to-eat fish 60 ham 40 chicken sandwich 40 vegetable sandwich 50 meat sandwich 50 minced meat	15% positive ready-to-eat fish 8.33% positive ham 5% positive chicken sandwich 45% positive vegetable sandwich 20% positive meat sandwich 32% positive minced meat	Ghorbani <i>et al</i> ^[79]
2017	Hamburger and minced meat	Culture and nested PCR	80 hamburger 70 minced-meat	1.42% positive hamburger 12.5% positive minced-meat	Gilani <i>et al</i> ^[76]

and 9.56%^[81] of the vegetables and traditional salads analysed.

The high prevalence of *H. pylori* in ready-to-eat foods, meats, milks and vegetables could be due to post-

processing contamination. In fact, the high prevalence of *H. pylori* in healthy human carriers^[11] suggests that foodstuff contamination due to poor hygiene management during milking, chilling and storage and during the handling, preparation and packaging of ready-to-eat foods may occur. Furthermore, *H. pylori* strains isolated from foods showed genotypes of *vacA* alleles similar to those in isolates from human clinical samples, endorsing the hypothesis that foods can be the source of *H. pylori* transmission to humans^[76,78-80].

However, the existence of animal reservoirs of the microorganism cannot be excluded^[65,66]. In addition, the histopathology of lesions by *H. pylori* in humans differs from that of many other gastric helicobacters, causing mild or absent inflammatory responses in their natural hosts^[82]. These data suggest that *H. pylori* may have not originally evolved as a human pathogen but was likely introduced into the human population from a mammalian reservoir sometime in the distant past^[65]. This hypothesis is further supported by evidence of *H. pylori* in the gastric mucosa of calves, pigs and horses^[83] and its isolation from sheep gastric tissue and milk^[65,84]. Furthermore, in the studies of Papiez *et al.*^[85] and Dore *et al.*^[65], *H. pylori* prevalence was higher in shepherds with direct animal contact than in controls without contact with sheep. Considering the 100% positive 13C-urea breath test in sheep, it may be reasonable to assume that these animal species may act as reservoirs and spreaders of *H. pylori*^[85,86]. However, further epidemiological and experimental studies are needed to corroborate these few data.

EVIDENCE SUPPORTING THE ROLE OF WATER IN THE TRANSMISSION OF *H. PYLORI* TO HUMANS

In the last Joint Monitoring Report (JMP) of 2017, "Progress on Drinking Water, Sanitation and Hygiene" by WHO and UNICEF^[87], the first global assessment of safe drinking water and sanitation services was reported. In 2015, approximately 2.1 billion people did not manage water safely, and among them, 844 million did not even have basic drinking water services, spending more than 30 min per trip to collect water from external sources, and some of them still drank untreated water from surface water sources such as streams and lakes. Globally, at least 2 billion people use a stool-contaminated source of drinking water. Contaminated water can transmit diseases such as diarrhoea, cholera, dysentery, typhoid and polio^[87].

It has been estimated that *H. pylori* colonizes more than half of the world's population, and contaminated water is mentioned as one of major causes^[60,88-91].

Bellack *et al.*^[92] developed a conceptual model of the role of water in *H. pylori* transmission. The hypothesis is that both humans and animals are long-term hosts and that water is a relatively short-term reservoir. *H. pylori*

may survive in water for a period before it is ingested as drinking water, accidentally during bathing, or through other pathways involving food. The infected person will spread *H. pylori* through faeces; through direct faecal-oral transmission, an infected person can infect another person or contaminate water bodies through direct contamination with faeces or indirectly with wastewater that comes into contact with the water used to drink. Animal contamination of water reserves may occur by defecating directly in surface waters or by faeces penetrating groundwater. The type of soil and heavy rain events can play an important role because they can facilitate the penetrability of manure containing bacteria in groundwater^[92].

Several epidemiological studies have been conducted on the transmission of *H. pylori* through water, and several risk factors have been highlighted, such as living in a house with a lack of internal water pipes, the use of well or river water, the use of a latrine, less frequent boiling of drinking water and little hand washing after use of the bath and before meals^[93-96] (Figure 1).

In 1991, a survey carried out on 407 Peruvian Lima children aged 2 mo to 12 years from families with different socioeconomic statuses showed an overall *H. pylori* prevalence of 48%. The children underwent the 13C-urea breath test, and the results showed a higher incidence among the children of low-income families than among those of high-income families (56% vs 32%). An important risk factor was the water supply; incidence increased three-fold when the water sources were outside the home compared to those whose homes had internal water sources. Furthermore, the municipal water supply seemed to be an important source of infection among Lima children from families of both low and high socioeconomic status because children from high-income families whose homes had municipal water were 12 times more likely to be infected than were those from high-income families whose water supplies came from community wells^[97].

The poor basic hygiene conditions and the lack of potable water have been reported as the cause of *H. pylori* infection in an epidemiological study of a population in a rural area of the state of Mato Grosso in Brazil. The survey was conducted on 40 children and adolescents and 164 adults. *H. pylori* antibodies were detected in 31 (77.5%) children and adolescents and in 139 (84.7%) adults. The most important identified risk factor is using untreated water that could be contaminated by wastewater due to the lack of a sewage system^[98].

Nurgalieva *et al.*^[49] conducted a similar study on 233 adults and 55 children in Kazakhstan. The overall prevalence of *H. pylori* was 86% among adults and 64% among children. The prevalence of *H. pylori* infection was inversely correlated with the index of clean water (CWI) (boil water before consumption, frequency of recovery and reuse of water and frequency of bath and shower). Infection was significantly lower among those

with a high CWI (56%) than those with a moderate (79%) or low (95%) CWI. Moreover, the prevalence of *H. pylori* was inversely related to socioeconomic status. Those living in a family in which the levels of education and study were low had a higher rate of *H. pylori* infection (90%) than did those from a higher socioeconomic group (69%)^[49].

In another epidemiological survey conducted in Germany on 3347 children from cities and rural areas, 179 children (119 from cities and 60 from rural areas) were infected by *H. pylori*. Among the children from rural areas, positivity significantly increased with the consumption of water from non-municipal sources^[99].

Fujimura *et al.*^[100] studied the presence of *H. pylori* in 4 Japanese rivers and in 224 children who lived near one river using the stool antigen test for *H. pylori* prevalence.

The results of this study showed that *H. pylori* DNA was frequently present in river water from the middle and downstream reaches in which the human biosphere is embedded. The author concluded that river water in the natural environment could be a risk factor for *H. pylori* transmission.

Occurrence of *H. pylori* in water

Despite the several epidemiological studies that support the hypothesis that *H. pylori* is a waterborne pathogen, the real role of water in the spread of the pathogen remains a topic of discussion. As with foodstuffs, the fastidious nature of the bacterium and the difficulties in isolating it from environmental sources do not provide unequivocal evidence about the role of water as a source of transmission of this microorganism.

Culture methods, immunological methods and molecular methods have been employed to detect *H. pylori* in the aquatic environment.

Several studies on the occurrence of *H. pylori* in sewage and drinking water samples have been carried out worldwide using molecular methods. In many of these surveys, it was not possible to isolate the bacterium using culture methods (Table 3).

Based on the findings that there is an association between water sources and the prevalence of *H. pylori* infection in Peruvian children^[97], *H. pylori* DNA was detected in drinking water samples from different locations near Lima (Peru) in two different surveys using molecular methods. These results provided evidence of the presence of *H. pylori* DNA in drinking water in Peru and were consistent with conclusions from a previous epidemiological study of the same population^[101,102]. In addition, other studies have highlighted the presence of *H. pylori* DNA in samples of tap water, well water^[103], aquatic systems located in Mexico City^[104], trucks for water transport and lake water^[105], further supporting the hypothesis of the transmissibility of *H. pylori* through water.

Despite the high incidence of *H. pylori* DNA in water, only a few studies have reported bacteriological isolation

of this microorganism. Bacteriological isolation of *H. pylori* occurred in the study of Lu *et al.*^[106], who analysed untreated municipal wastewater samples using a series of steps beginning with immunomagnetic separation and cell culture.

In a survey carried out in Iraq, out of 198 samples of treated municipal drinking water, 10 strains of *H. pylori* were isolated and identified. The low concentration of chlorine in the water samples and the ability to form biofilms in water pipes^[107,108] were the reasons that *H. pylori* was isolated^[109].

H. pylori was also isolated in tap water, dental unit water, and bottled mineral water in Iran. Out of 200 water samples collected in Iran, 5 cultures were positive. Two out of 50 tap water samples (4%), 2 out of 35 dental unit water samples (5.8%), and 1 out of 40 samples (2.5%) from water coolers in public places were found to be contaminated with *H. pylori*^[110]. Ranjbar *et al.*^[111] examined 450 bottled mineral water samples and confirmed the presence of *H. pylori* in bottled mineral water.

Survival studies in water samples showed that *H. pylori* could be cultured from 48 h up to 20 d in autoclaved distilled water. An increase in survival occurs at low temperatures; in fact, high temperature causes loss of culturability^[89,91,112-118]. Shahamat *et al.*^[118] used an autoradiographic method to detect the metabolic activity of *H. pylori* VBNC in water. Four *H. pylori* strains were studied using 72-h cultures in water and incubated with [3H] thymidine for 24-72 h. After being exposed to the Kodak NTB2 emulsion for 3-28 d, the organism was vital and culturable under these conditions for up to 48 h and, in some cases, 20 to 30 d (Table 3).

One factor in support of *H. pylori* infection being waterborne or related to poor health practices is the association, which some authors claim, of *H. pylori* with free-living amoebae (FLA), such as *Acanthamoeba*, *Naegleria*, *Vermamoeba* or *Balamuthia*, which are ubiquitous protozoa commonly found in water^[119-122].

Several studies have also shown that *H. pylori* can be present as a biofilm on the pipes of the drinking water system with the ability to adhere to different hydraulic materials, such as copper and stainless steel^[121,123,124]. As a result, *H. pylori* is likely to survive in an aquatic system within a biofilm rather than in the planktonic state^[39,125].

H. pylori cells were able to survive for short periods in chlorinated drinking water in the VBNC form, which would allow them to reach final consumption points and, at the same time, enable them to be undetectable by culture methods^[115].

Moreover, in biofilms, the resistance of *H. pylori* to chlorine increases significantly^[120,126]. Therefore, it is possible that if the organism enters a distribution system, it may survive disinfection treatment within the biofilm matrix^[115]. This characteristic may be based on the lack of isolation in some surveys of *H. pylori*. In fact, in water samples treated with suitable potabilization

Table 3 Studies evaluating the occurrence and survival of *Helicobacter pylori* in water

Year	Water and study type	Method	Observations	Ref.
1993	Survival of <i>H. pylori</i> in artificially contaminated sterile river water	Culture	Culture up to 48 h	Shahamat <i>et al</i> ^[118]
1996	Occurrence of <i>H. pylori</i> in 48 water samples: 30 from municipal water system, 14 from community taps, 4 from brick tanks or plastic barrels of different households	IMS and PCR	50% PCR positive samples	Hulten <i>et al</i> ^[101]
1997	Study on <i>H. pylori</i> resistance to chlorination	Culture	<i>H. pylori</i> were readily inactivated by free chlorine	Johnson <i>et al</i> ^[117]
1999	Occurrence of <i>H. pylori</i> in water from rivers and ponds	IMS and nested PCR	<i>H. pylori</i> -specific DNA was detected in samples	Sasaki <i>et al</i> ^[103]
1999	Occurrence of <i>H. pylori</i> in water from delivery truck and two lakes	Nested PCR and Southern blot hybridization	PCR positive samples from truck	McKeown <i>et al</i> ^[105]
2001	Occurrence of <i>H. pylori</i> in 10 seawater samples, 10 river water samples, 10 tap water samples, 6 well water samples	IMS, real-time PCR and nested PCR	2 PCR positive samples of well water	Horiuchi <i>et al</i> ^[127]
2001	Occurrence of <i>H. pylori</i> in 139 ground water samples	PCR and Southern blot hybridization	69% positive samples	Mazari-Hiriart <i>et al</i> ^[104]
2002	Occurrence of <i>H. pylori</i> in raw municipal wastewater	IMS, culture and PCR	23 out of 37 isolated strains were confirmed to be <i>H. pylori</i> 11 out of 23 strains of <i>H. pylori</i> demonstrated <i>vacA</i> gene heterogeneity	Lu <i>et al</i> ^[106]
2002	Study on the susceptibility of <i>H. pylori</i> to chlorine, monochloramine, and ozone compared to that of <i>Escherichia coli</i>	Culture	<i>H. pylori</i> was more resistant than <i>E. coli</i> to chlorine and ozone but not monochloramine	Baker <i>et al</i> ^[116]
2004	Occurrence of <i>H. pylori</i> in water and biofilms: 11 samples from domestic proprieties, 7 samples from educational properties and from hydrants, and samples from reservoirs and water meters of 3 water utilities	Culture, IMS and PCR	All cultures were negative 26% PCR positive sample with the highest frequency in biofilm	Watson <i>et al</i> ^[120]
2004	Occurrence of <i>H. pylori</i> in seawater	Nested-PCR	<i>H. pylori</i> DNA only detected in fractionated water samples containing zooplanktonic organisms	Cellini <i>et al</i> ^[130]
2005	Occurrence of <i>H. pylori</i> in seawater	Filtration (200 mm, filter), culture and PCR	<i>H. pylori</i> was only isolated from fractionated water samples containing large zooplanktonic organisms	Cellini <i>et al</i> ^[131]
2005	Occurrence of <i>H. pylori</i> in 36 seawater samples	Culture and PCR	30 positive samples	Carbone <i>et al</i> ^[132]
2006	Study on the ability of <i>H. pylori</i> to adhere on different water-exposed abiotic surfaces	Scanning electron microscope	<i>H. pylori</i> was able to adhere to all substrates tested	Azevedo <i>et al</i> ^[123]
2007	Study on the ability of <i>H. pylori</i> to adhere to stainless steel 304 in different environmental conditions	Epifluorescence microscopy	<i>H. pylori</i> was able to adhere to stainless steel 304	Azevedo <i>et al</i> ^[124]
2007	Study on the resistance of <i>H. pylori</i> to chlorination	Culture, FISH, PCR and RT-PCR	Culture until 5 min FISH viable cells until 3 h PCR samples positive after 24 h RT-PCR positive after 24 h	Moreno <i>et al</i> ^[115]
2007	Survival of <i>H. pylori</i> in spiked bottled mineral water (drinking water)	Culture epifluorescence microscopy and PCR	Culture until 5 d Cell viability until 14 d	Queralt <i>et al</i> ^[114]
2007	Survival of <i>H. pylori</i> in spiked chlorinated filtered water (drinking water)	Culture, FISH and PCR	Culture until 5 min FISH viable cells until 3 h PCR positive after 24 h RT-PCR after 24 h	Monero-Mesonero <i>et al</i> ^[115]
2009	Occurrence of <i>H. pylori</i> in 75 drinking and environmental water samples and 21 natural water biofilms samples	Real-time PCR	0% positive samples	Janzon <i>et al</i> ^[129]
2010	Occurrence of <i>H. pylori</i> in 198 drinking water samples	Culture	10 out of 469 isolated strains were confirmed <i>H. pylori</i>	Al-Sulami <i>et al</i> ^[109]
2011	Occurrence of <i>H. pylori</i> in 137 seawater samples	PCR	21% of the samples were positive for <i>H. pylori</i>	Twing <i>et al</i> ^[134]

2013	Occurrence of <i>H. pylori</i> in 50 tap water samples, 35 dental units' water samples, and 40 bottled mineral water samples	Culture and PCR	2 positive tap water samples 2 positive water from dental unit samples 1 positive water coolers sample	Bahrani <i>et al.</i> ^[110]
2013	Occurrence of <i>H. pylori</i> in 31 seawater samples	Culture and PCR	4 positive samples	Holman <i>et al.</i> ^[135]
2016	Occurrence of <i>H. pylori</i> in 450 bottled mineral water samples	Culture and PCR	8 positive samples	Ranjbar <i>et al.</i> ^[111]
2018	Occurrence of <i>H. pylori</i> in 241 drinking water samples	PCR	49 positive samples	Boehnke <i>et al.</i> ^[102]

H. pylori: *Helicobacter pylori*.

systems, the lack of isolation can be caused by the formation of the biofilm or by the conversion of *H. pylori* in the form of VBNC^[127-129].

Some important clues have emerged from Italian research on the presence of bacteria in seawater. Work by Cellini *et al.*^[130,131] and subsequent investigations^[132] suggest that a significant reservoir for the microorganism is seawater, in which *H. pylori* can occur both in a free-living form and in association with plankton. Plankton-related *H. pylori* cells were detected in both summer and winter months depending on the flowering of Copepods and Cladocerans. The authors supposed that zooplankton organisms represent a sort of protected niche for survival of the microorganism. The finding of *H. pylori* attached to planktonic organisms is particularly interesting for the role of the latter in the seafood chain and its subsequent potential role in the spread of *H. pylori* infection^[130-132]. More generally, the presence of *H. pylori* in seawater could also be a health hazard for swimmers and others using those waters for work or pleasure^[133].

Moreover, *H. pylori* DNA has been isolated in 21% of samples from freshwater, estuarine and beach sites in Delaware (United States)^[134] and in seawater sampled from 31 locations in Georgia, Trinidad and Puerto Rico^[135]. In both reports, no correlation between the occurrence of *H. pylori* and faecal indicator bacteria was found, suggesting that standard water quality tests are ineffective in predicting the presence of this pathogen in natural waters, confirming the potential risk for *H. pylori* presence in marine waters.

METHODS FOR THE DETECTION OF *H. PYLORI* IN FOODS AND WATER

The isolation of *H. pylori* from food samples, particularly when they present high loads of accompanying microflora, is demanding and time consuming because it requires the use of selective media with numerous antibiotics, microaerophilic conditions and long incubation periods (7 d)^[55,71]. The detection of *H. pylori* in food samples and water by means of conventional microbiological techniques generally employed for clinical specimens, which are unable to detect the VBNC, may yield false negative results and thus underestimate the presence of the bacterium in food; furthermore, the presence of *H. pylori* in VBNC state in food and water represents a

potential microbiological risk for consumers, especially as a source of virulence factors^[37,136,137].

Several solid and liquid culture media for the selective isolation of *H. pylori* from foods have been tested. The culture media most suitable for *H. pylori* growth often contain defibrinated horse or sheep blood acting as a reducing agent^[29]. Furthermore, to achieve replication of *H. pylori* in broth culture, agitation is mandatory to provide good dispersion of gases throughout the liquid^[31].

Brain heart infusion broth (BHIB) with growth supplement and selective agents has been evaluated^[26,56]. BHIB with horse serum supplemented with porcine stomach mucin (0.3%), ferrous sulphate and sodium pyruvate (5%) or urea, along with the adjustment of the pH to 5.5 or 4.5, enhances the survival and possibly enables the growth of *H. pylori* in enrichment medium with fresh ground beef. In particular, pH 5.5 greatly enhances the growth and detectability of *H. pylori* in foods and should be considered an important factor for the detection of *H. pylori* in enrichment culture^[56]. Stevenson *et al.*^[55] compared the growth of *H. pylori* in several liquid and solid media. None of the media tested presented an outstanding performance; only *H. pylori* special peptone agar offered the advantage of allowing the formation of the largest *H. pylori* colonies, and it was suitable for recovering *H. pylori* from environmental samples likely to be contaminated with large numbers of competing microorganisms^[31,55].

Poms and Tatini^[26] evaluated the efficacy for the *in vitro* isolation of *H. pylori* from foods of two solid media containing tryptic soy agar and Wilkins-Chalgren anaerobe agar supplemented with 5% defibrinated horse blood. The latter, to which several antibiotics (30 mg/L colistinmethanesulphonate, 100 mg/L cycloheximide, 30 mg/L nalidixic acid, 30 mg/L trimethoprim, and 10 mg/L vancomycin) were added, was found to be highly selective for the recovery of *H. pylori* from foods, but it lacked sufficient sensitivity to detect very low numbers of the bacterium.

Many authors have successfully used Wilkins-Chalgren anaerobe-agar or the broth developed by Poms *et al.*^[26] for the isolation of *H. pylori* in foods, both supplemented as described above^[70,76,78-80].

However, there are still no standardized isolation protocols that are able to isolate the few *H. pylori* cells

present in samples rich in microbial flora such as food. Furthermore, the pathogen is able to enter a VBNC state that remains metabolically active but fails to develop into colonies when cultured on routine media^[40]. Consequently, molecular assays have been conducted to detect *H. pylori* DNA in water and foodstuffs.

Immuno-separation (IMS) followed by PCR has been successfully used by several investigators^[138-140]. The advantage of using this protocol is that it offers excellent specificity using the IMS able to concentrate the pathogen from foods, followed by high sensitivity of the molecular methods^[29]. Nevertheless, it appears expensive, exacting and time consuming. Autoradiography and ATP bioluminescence have been successfully used for the detection of *H. pylori* from water, human stools and pure cultures but have never been tested on food^[29,118]. In addition, the ATP bioluminescence assay does not allow for distinguishing among ATP from different cell sources when applied to a complex system such as a foodstuff^[141].

A multiplex touchdown PCR (MT-PCR) method for the identification (16S rRNA gene) and genotyping (*vacA*- s1/m1, s1/m2, and s2/m2- and *cagA* genes) of *H. pylori* directly from artificially contaminated sheep milk was developed^[142]. The characterization of the genes encoding virulence factors provides important information with respect to the sanitary assessment of food items because of the greater pathogenicity of certain *H. pylori* genotypes. Hence, for public health purposes, the evaluation of a food containing *H. pylori* will thus have to include the genotyping of isolates. This rapid, sensitive (15 cfu/mL) and specific molecular method presents the advantage of detecting and genotyping *H. pylori* from microbiologically complex foodstuffs in a single step^[142]. A nested PCR approach has been employed for the detection of the *H. pylori glmM* gene from seawater and sheep, goat and cow milks^[71,130,141]. The sensitivity of the nested PCR technique was 3 cfu/mL in all types of milk and 62 cfu/mL in seawater samples, and therefore, compared to the previously described MT-PCR, it was more sensitive for the detection of *H. pylori* from foods (3 cfu/mL vs 15 cfu/mL) with the same specificity^[141]. Osman *et al.*^[73] employed nested PCR in 50 cow milk samples, and 22% were positive for the presence of the *H. pylori glmM* gene.

H. pylori, as with many other bacteria, is able to form biofilms within which it can survive due to the different protection mechanisms that the biofilm offers. Quantitative real-time PCR was developed for the detection of *H. pylori* in drinking water biofilms of different ages. The target gene was the *ureA* subunit of the *H. pylori urea* gene, which showed high specificity and sensitivity^[143].

As is well known, the main limit of PCR assays is their inability to distinguish live organisms from dead organisms. PCR techniques can, however, be used to screen water and foodstuffs, thus making it necessary to use conventional isolation methods only on those samples that test positive by PCR.

In a study by Buck *et al.*^[58], mRNA of known virulence factor (*vacA*) was detected in VBNC *H. pylori* cells using RT-PCR. This method exploits the unstable nature of bacterial mRNA to infer pathogenic viability when *H. pylori* becomes non-cultivable^[144]. The half-life of mRNA of *E. coli* and *Vibrio vulnificus* cells is approximately 3-8 min and less than 60 min, respectively^[145,146]. Thus, mRNA can be an excellent indicator of viability when *H. pylori* occurs in the VBNC state. Moreover, detection of transcripts from the *vacA* virulence gene may deduce continued virulence activity of *H. pylori* when present in the VBNC state^[58]. This molecular technique offers significant promise for the detection of microorganisms in water and foodstuff and is a valid alternative to culture methods.

The fluorescence in situ hybridization (FISH) assay with the rRNA-direct molecular method has been applied for the specific detection of *H. pylori* in river and wastewater samples^[147] and in raw bulk tank bovine milk^[67] and for the assessment of its survival in chlorinated drinking water^[115,148]. The authors concluded that FISH was a rapid method for the direct detection and specific identification of viable bacteria in food^[67].

CONCLUSION

Several studies report the presence and survival of *H. pylori* in foods and water, especially in milk and in ready-to-eat products, suggesting that they can be sources of infection for humans.

Although many of the findings reported in the literature are based on indirect evidence of *H. pylori* in food and water through molecular methods and there are only in a few cases on the bacteriological isolation of this microorganism, the possibility that food and water can be routes of transmission among others cannot be ruled out.

Most of the bacteriological isolations of the pathogen in foods and water have been obtained in work conducted in Iran; a possible explanation could be the greater prevalence of the disease in this geographical area than in other areas. Moreover, the discrepancy in the prevalence of *H. pylori* in the different surveys could be related to the type and number of samples tested, sampling method, experimental methodology and climate differences in the regions from which the samples were collected.

However, to confirm a definite foodborne and waterborne role of *H. pylori* transmission, more surveys are needed on the presence of *H. pylori* in other foods of animal origin, particularly in seafood, and on the survival ability of this microorganism in dry fermented sausages and dairy products. Further investigations on the possible role of humans and animals as reservoirs of the microorganism are also required to clarify the faecal-oral route of transmission and the method of food and water contamination. Finally, the development of molecular biology methods and, above all, bacteriological isolation methods of *H. pylori* from

water and food would add provide data that could confirm or deny the role of *H. pylori* as a foodborne and waterborne pathogen.

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