REVIEW ARTICLE
A conceptual model of water's role as a reservoir in *Helicobacter pylori* transmission: a review of the evidence

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SUMMARY

Helicobacter pylori infection plays a role in the development of chronic gastritis, peptic ulcer and gastric cancer, yet the route of transmission into susceptible hosts remains unknown. Studies employing microbiological techniques have demonstrated that *H. pylori* has the ability to survive when introduced into water and that *H. pylori* is present in water and other environmental samples all over the world. Epidemiological studies have shown that water source and exposures related to water supply, including factors related to sewage disposal and exposure to animals, are risk factors for infection. This review describes the microbiological and epidemiological evidence for, and proposes a model of, waterborne *H. pylori* transmission outlining important features in the transmission cycle. In the model, humans and animals shed the bacteria in their faeces and the mechanisms for entry into water, and for survival, ingestion and infection are dependent upon a range of environmental influences. Verification of the proposed model pathways has important implications for public-health prevention strategies.
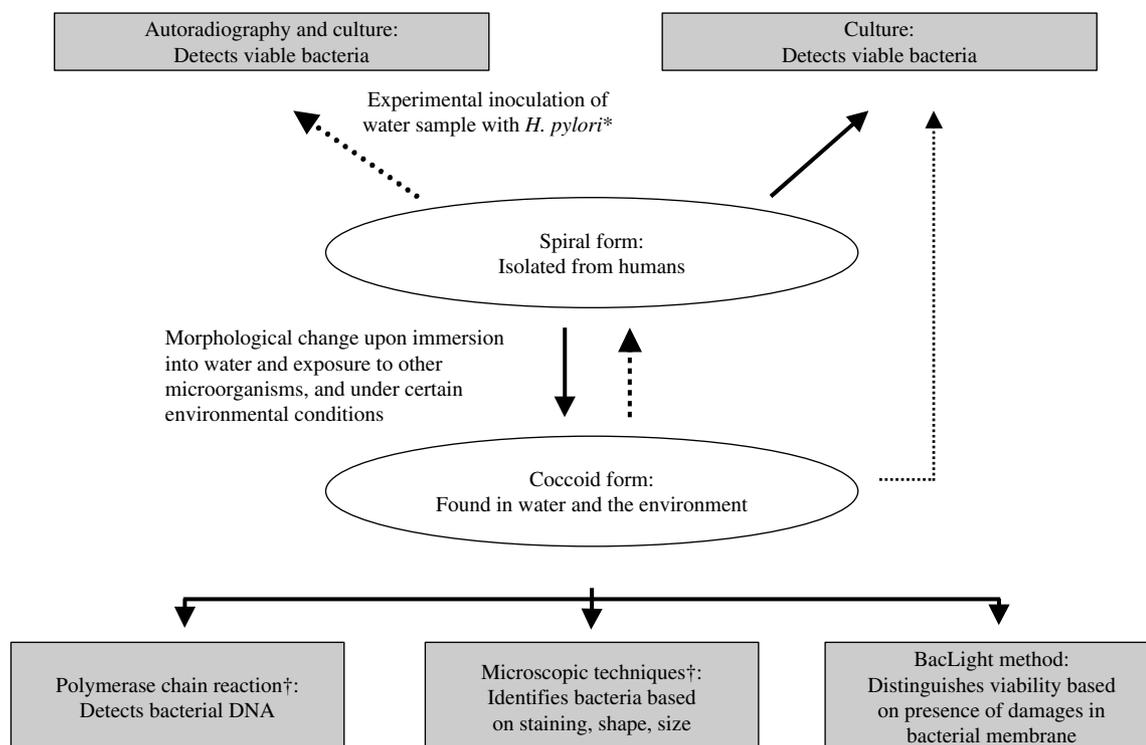
INTRODUCTION

The isolation of the bacterium *Helicobacter pylori* and subsequent findings of its role in the development of chronic gastritis, peptic ulcers and gastric cancer represented major medical breakthroughs. These discoveries have had a considerable impact on reducing the burden of gastrointestinal ailments. Current treatments aim to eradicate *H. pylori* infection using antibiotics, whereas previous treatments involved surgery or erroneously focused on stress reduction, dietary changes, and antacid therapies which often resulted in recurrence of symptoms [1, 2].

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It is estimated that over half of the world's population are carriers of *H. pylori*, making it one of the most common bacterial infections in the world [3]. However, many individuals harbouring the bacteria never develop clinical symptoms. Differences in outcome are thought to result from a variety of factors including age at acquisition, exposures to other environmental agents, and genetic characteristics of both host and *H. pylori* bacterium [4]. Although the incidence of *H. pylori* infection and peptic ulcers and gastric cancer has decreased over the past century, it still represents a major burden [5]. Worldwide, there are an estimated six million incident cases of duodenal ulcer each year and 900 000 cases of gastric carcinoma [6].

There are two distinct patterns of *H. pylori* acquisition. In developing countries, most people are infected as infants or pre-adolescents and remain

* Possible morphological into coccoid form; † Unable to distinguish viable from non-viable forms

Fig. 1. Methods of *H. pylori* detection. ——, Strong evidence in published literature; ▪▪▪▪, some evidence in published literature; ⋯⋯, insufficient evidence.

infected for the duration of their life whereas in developed countries it appears that infection is acquired gradually with increasing age [7]. The higher overall prevalence of infection in developing countries suggest that hygiene and environmental factors may play a significant role in transmission, a theory supported by data showing lower socioeconomic status as a predictor of infection within defined populations [8, 9].

While the major health outcomes of *H. pylori* infection are well documented, routes of transmission remain unclear, making it difficult to implement public health measures to prevent infection. Primary prevention is particularly important as antiobiotic-resistant strains exist and further antibiotic use may give rise to increased resistance among *H. pylori* and other bacteria [10]. Aside from iatrogenic modes of transmission, person-to-person pathways have been proposed via faecal–oral or oral–oral routes [11]. Water may be an intermediate in faecal–oral transmission by acting as a reservoir in which the bacteria can remain for periods of time before it is ingested as drinking water, accidentally during bathing, or through other pathways involving food. The purpose

of this paper is to review the microbiological and epidemiological evidence to determine what is currently known about the feasibility of water as a reservoir for transmission. A conceptual model of possible transmission pathways is proposed which incorporates this evidence and provides direction for future research on the risk of transmission associated with environmental factors.

A literature search was conducted in the following databases: (1) Medline, (2) Web of Science and (3) Environmental Sciences and Pollution Management for articles published from 1985 to the present. A combination of the following keywords were used: 'Helicobacter pylori', 'water', 'faecal/fecal', 'transmission', 'epidemiology' and 'microbiology'. Abstracts were read by the principal author and papers selected if they presented original research investigating (1) environmental factors associated with *H. pylori* infection or transmission; (2) other factors related to environmental transmission of *H. pylori* through water systems or sources; (3) methods used to detect *H. pylori* in the water supply, or, (4) environmental sampling for *H. pylori* in the natural environment. A total of 80 articles met the
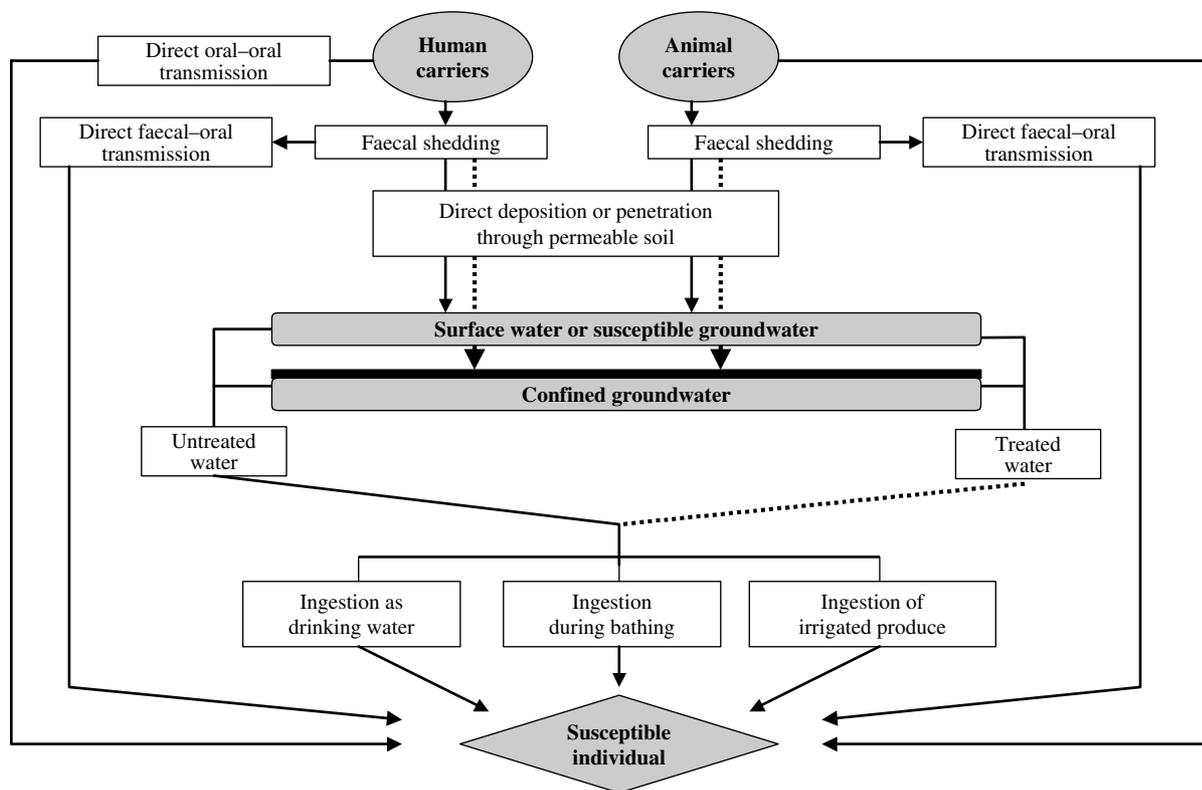
Fig. 2. Conceptual model of waterborne *H. pylori* transmission pathways. ⬭, Long-term host; ▭, short-term reservoir; ──, transmission pathway; ▪▪▪▪, reduced transmission probability pathway.

inclusion criteria and were reviewed for this paper for evidence to inform a hypothesized conceptual model of water as a reservoir in *H. pylori* transmission.

DETECTION OF *H. pylori* IN WATER AND THE ENVIRONMENT

Over the past 15 years, substantial attention has been paid to the development of methods to detect *H. pylori* in water. These have been employed in a variety of settings and have contributed to the hypothesis of waterborne transmission. Figure 1 presents an overview of these methods and demonstrates that viable *H. pylori* has not been cultured from environmental sources. However, sufficient evidence does exist to formulate a conceptual model of how water may be involved in the transmission pathway. Below is a summary of the evidence leading to these proposed pathways and the hypothesized conceptual model is presented in Figure 2.

Methods of detection

Culturing *H. pylori* from areas outside the human stomach has been difficult because of a morphological change in the bacterium and overgrowth by competing microorganisms [12, 13]. Attempts to culture the bacteria from inoculated water samples have been made using alternative growth media under variable conditions [14, 15]. The most common detection techniques are the polymerase chain reaction (PCR), autoradiography, or microscopic investigation of a stained sample. Sensitivity of these methods is estimated by testing *H. pylori*-inoculated samples while specificity is determined by testing samples inoculated with other *Helicobacter* organisms and structurally similar bacteria.

Experimental studies

Studies in controlled laboratory settings have revealed some information about the behaviour of *H. pylori* in the aqueous environment. Generally, these studies involve the inoculation of aqueous samples with *H. pylori* at different concentrations and under different conditions and follow-up tests are carried out to determine the effect on survival.

Survival times as long as 20 days have been reported in distilled water [16]. Increased survival times are associated with lower temperatures [16–19],

pH levels of 5·8–7·0 [20], and milk and saline solutions compared to water [17, 21]. Survival may be dependent on even more specific conditions of the aqueous environment including the presence of other micro-organisms such as zooplankton and free-living amoebae [22, 23]. Experimental work has also shown that *H. pylori* can incorporate into biofilms on surfaces in aqueous environments which is important since growth in water is probably negligible if it occurs at all and the biofilms may provide a substrate that would allow multiplication to occur [24–26]. The addition of chlorine to water supplies has proven effective at inactivating *H. pylori* [27].

Overall, these findings indicate that if introduced into an aqueous environment, *H. pylori* can survive for periods of time and the length of that time is affected by a number of specific conditions of the aqueous environment.

Detection in the natural environment

Experimental studies have enabled the development of detection methods and demonstrated the possibility of water serving as a reservoir for *H. pylori*. PCR and microscopic techniques have been employed for the examination of environmental samples taken from all over the world.

H. pylori has been detected in wastewater systems, which is not surprising given that carriers are known to shed the bacteria in their faeces [28, 29]. However, positive results in both pre- and post-treated wastewater suggests that wastewater treatment may be ineffective in removing *H. pylori* from this aqueous environment [29]. *H. pylori* has been detected in surface water and groundwater samples in various parts of the world, in some places where human or animal faecal contamination was likely and in water intended for human consumption in both developing and developed parts of the world [28–33]. *H. pylori* DNA detected in water pots and in a cast-iron water pipe from a municipal water system suggests that biofilms may play a role in transmission in real-world settings [34, 35]. Zero percent prevalence in chlorine-treated drinking water offers further evidence that it may be an effective method for disinfection [29].

The identification of *H. pylori* DNA on flies and in cow faeces, and in raw and pasteurized milk samples illustrates that animals may directly or indirectly play a role in zoonotic-related water transmission [31, 36]. The presence of *H. pylori* in sheep with the absence of an immune response indicates that they may be

a natural host for the bacteria [37]. The isolation of *H. pylori* and other *Helicobacter* spp. from the stomachs of many animals, including dogs and cats, suggests that zoonotic transmission is a possibility. However, the role of non-*pylori* species in this process is unknown [38–41].

Generally, the detection of *H. pylori* in the environment has shown that water and water sources linked to zoonotic reservoirs are a possibility that should be considered in the hypothesized link between water and *H. pylori* infection. However, these results alone do not verify that they are involved in transmission. The viability of *H. pylori* once it enters water and its capability to colonize the human stomach upon ingestion are key components to solving the question of waterborne transmission.

Viability of *H. pylori* in water

Although *H. pylori* bacteria become non-culturable by traditional methods after entering water, they may exist in a viable but non-culturable (VBNC) state. *H. pylori* undergoes a morphological change from mostly spiral to mostly coccoid and U-shaped forms in water that probably contributes to the loss of culturability [16, 42, 43]. It has been suggested that transformation to the coccoid form is the manifestation of cell death and that it enables the bacterium to resist the potentially adverse effects of entering aquatic environments, which has also been observed with other bacteria [16, 44–46].

Determining the viability of *H. pylori* in water is challenging because the methods used to detect the bacteria in water and the environment are unable to distinguish between viable and dead bacteria [14]. Other factors such as the presence of yet unidentified *Helicobacter* spp. may account for some false-positive results for *H. pylori* detection [10]. Positive results using some primers but not others in PCR indicates that this may be occurring [47].

In light of this, a number of approaches have been taken to determine the viability of *H. pylori* in water. For example, although non-culturable, *H. pylori* was deemed viable after immersion in natural freshwater as determined by a LIVE/DEAD BacLight assay (Invitrogen Canada Inc., Burlington, Ontario, Canada) which distinguishes between living and dead bacteria on the basis of intactness of cell membranes [48, 49]. Another approach is to culture for a limited period of time, which prevents complete overgrowth of competing bacteria, followed up by PCR [13].

Based on these and other findings, it seems likely that *H. pylori* passes through a VBNC state for a brief period before proceeding to cell death [50]. As demonstrated in previous studies, it is likely that the speed of the conversion process is affected by the aqueous environment and the presence of other organisms in the water [22, 48].

However, the question remains as to whether this non-culturable form is able to colonize. The ability of the coccoid form to colonize has been tested using animal models, although results have been contradictory. Successful colonization was observed in mice after intragastric colonization with the coccoid form, however, colonization was unsuccessful in piglets [51, 52]. Experiments with other bacteria have indicated that they can maintain their pathogenicity in the VBNC state and are able to multiply after a dormant phase thereby making colonization possible [53–56].

In summary, it has been shown that the presence of *H. pylori* has been detected in the environment with various tests; however, it has not been convincingly cultured from naturally occurring samples. Isolation from the environment by means of culture would demonstrate that viable *H. pylori* is present outside the human stomach and further strengthen the theory of waterborne transmission.

THE EPIDEMIOLOGY OF WATER AND *H. pylori* TRANSMISSION

The epidemiology of *H. pylori* transmission has been studied extensively and numerous risk factors have been uncovered. This section reviews studies that have examined water source as a risk factor in *H. pylori* transmission in addition to other exposures that may be important in the water transmission pathway.

Water and water-related risk factors

Water source has been implicated as a risk factor for *H. pylori* infection in epidemiological studies in a variety of settings, mostly in developing countries. In different settings, water from different sources (i.e. municipal *vs.* private) is variable in quality because of the diversity in environmental influences and water distribution systems. For example, a study of Peruvian children found that those relying on municipal water were significantly more likely to have a positive urea breath test (UBT) than those using

community well water (OR 11·4, $P = 0·02$) [57]. In Lima, Peru, municipal water is taken from the Rimac River, which is heavily contaminated by industrial and domestic faecal pollution. A study in Germany found that children drinking from non-municipal sources were at an increased risk of infection compared to those relying on municipal water supplies (OR 2·8, 95% CI 1·0–8·2, $P < 0·05$) [58]. In Germany, the municipal water supply is generally thought of as the cleaner water supply and, although private wells exist and are used for economic reasons, there is a ban on drinking untreated water [59]. Other surveys indicate that the prevalence of infection is higher among people drinking untreated water [60] or water from shallow wells [61], and among people lacking tap water or an internal water supply in the home [57, 62–65].

Findings from other studies strengthen the possibility that water plays a role in *H. pylori* transmission. In a rural area of The Gambia, infants enrolled in a cohort study at a time when the deep bore-hole water supply was disrupted had a median age of first positive UBT of 8 weeks, while those recruited when the water supply was re-established had median first positive UBT at 28 weeks [35]. A cross-sectional survey in Kazakhstan found that people with a high clean water index (CWI) (based on frequency of boiling water, storing and reusing water and frequency of bathing) were less likely to have a positive UBT than those with a medium (OR 1·9, 95% CI 1·3–3·1) or low CWI (OR 5·1, 95% CI 1·8–20·5) after adjustment for age and socioeconomic status [63]. The importance of water storage has been demonstrated in a study in rural Bolivia when fewer seroconversions were observed for children whose families used a narrow-mouthed water vessel with a lid compared to those using the traditional, open-mouthed water vessel (OR 0·4, 95% CI 0·2–0·9) [66]. Frequency of swimming in streams and other waterways susceptible to contamination are also noted risk factors for infection [61, 67].

The presence of *H. pylori* DNA as detected by PCR in water close to human-impacted areas may be an indication of faecal contamination and suggest the importance of faecal–oral transmission [68]. Evidence of this can be found in relationships between infection and the presence and type of sewage system. People with indoor bathrooms are generally less prone to infection [62, 63, 69, 70] while elsewhere the presence of a flush toilet is protective of *H. pylori* infection [65]. Although these findings may suggest that transmission occurs by direct faecal–oral contamination,

a closer look may be needed to investigate the interaction between sewage disposal and water, a feasible pathway for transmission that has not been thoroughly investigated in the literature.

Some studies suggest that other vehicles may also be involved in water-related *H. pylori* transmission. For example, the consumption of uncooked vegetables has been identified as a risk factor in different settings [67, 71]. Bacterial contamination may have originated from irrigation water containing faecal matter as *H. pylori* has been detected in water used for agricultural purposes in at least a few settings [29, 71]. The role of food in *H. pylori* transmission is discussed in more detail elsewhere [72].

Zoonotic transmission may occur with animals acting as carriers for *H. pylori*. A high prevalence of infection among shepherds compared to farmers offers evidence that sheep may pass on the bacteria [73]. The presence of pets has also been identified as predictors of infection in some situations [58, 74] but not in others [75, 76]. In some cases, the presence of pets is an indicator of elevated social status, while in others, it may exacerbate lower levels of hygiene. High levels of *H. pylori* antibodies found in workers exposed to freshly cut animals parts relative to those in clerical positions without animal exposures further suggests that *H. pylori* infection may be a zoonoses [77]. While direct contact with animals remains one possible mode of transmission, indirect transmission may occur when animals contaminate susceptible surface water or groundwater sources used for drinking water, which is more likely to occur in areas of high-density agricultural production.

Evidence against waterborne transmission

Epidemiological evidence against common-source transmission such as drinking water comes from studies finding a negative association between *H. pylori* infection and hepatitis A virus (HAV), which is used as a marker of exposure to faecal matter because it is transmitted via faecal–oral pathways [78–80]. However, low rates of co-infection do not eliminate the possibility of a common-source transmission as HAV is more contagious than *H. pylori*, HAV infection lasts a relatively short period of time, multiple routes of transmission may exist for each organism and the distribution of the two micro-organisms in the population may be different [81]. Non-significant differences in *H. pylori* prevalence of workers occupationally exposed and not exposed

to sewage [82, 83] and low rates of seroconversion among travellers to developing countries [84, 85] have led some to reject the notion of common-source pathways. However, this ignores the likelihood that people take special precautions to avoid potentially high-risk exposures while at work or travelling.

DIFFICULTIES IN INVESTIGATING WATERBORNE *H. pylori* TRANSMISSION

The principal challenge with conducting environmental sampling is the inability to demonstrate the existence of viable *H. pylori* in water that is able to then colonize upon entering the stomach or duodenum. Despite the detection of non-viable *H. pylori* using other methods, the concept of waterborne transmission is likely to remain in question until it is cultured from natural sources. However, we have presented microbiological and epidemiological evidence to suggest that water is a feasible transmission route for *H. pylori* infection that warrants further investigation from a public health perspective in the absence of methods to culture *H. pylori* from natural sources.

From an epidemiological perspective, many studies investigating *H. pylori* transmission face similar limitations to one another. In general, *H. pylori* transmission has been difficult to investigate because of the high proportion of asymptomatic chronic infection and the absence of a known indicator of recent *H. pylori* acquisition. Studies of children, especially in developing countries, may be biased because some non-invasive diagnostic tests are less accurate in children than adults and validation of diagnostic tests in the study population may be less common [86, 87]. The UBT is more reliable in children older than 6 years [88] and its use in studies of infants has yielded high rates of apparent spontaneous clearance [89], which is unlikely. Therefore, studies that use only a single UBT measurement to determine *H. pylori* carrier status may be inaccurate and in these situations, non-differential misclassification is likely to bias risk estimates towards the null.

In studies where drinking water is the exposure of interest, it is difficult to track all drinking water sources for an individual as they may rely on several sources in a single day and even water from a single source may be quite variable in quality over time. Other problems arise when understanding the importance of risk factors that may be relevant to more than one transmission pathway. For example,

Table. *Important factors in the hypothesized waterborne* H. pylori *transmission cycle*

Entry into water	Survival
• Proximity to human and animal impacted areas	• Length of time in water
• Penetrability of soil	• Water temperature
• Precipitation	• Water pH
• Susceptibility of water to contamination	• Presence of other microorganisms or suspended
• Presence of *H. pylori* in biofilm in water pipe	sediments
or container	• Water treatment
Ingestion	**Infection**
• Drinking water	• Viability of *H. pylori*
• Consumption of raw vegetables	• Susceptibility of host
• Swimming or bathing	

clustering patterns in families may be an indication of either oral–oral transmission via direct contact or the sharing of drinking glasses, or of faecal–oral transmission through a shared exposure to a specific water source.

WATERBORNE *H. pylori* TRANSMISSION MODEL

The plausibility of waterborne *H. pylori* transmission has been demonstrated by various microbiological and epidemiological studies. Based on this evidence, a conceptual model of waterborne transmission is presented in Figure 2. The most important assumption of this model is that humans and animals are long-term hosts and water is a relatively short-term reservoir in which *H. pylori* may remain for period of time before it is spread to susceptible humans through direct consumption of water containing the bacteria or indirectly through the consumption of contaminated food. A human carrier will spread the bacteria by shedding it in their faeces. At this point, the bacteria may come into contact with a susceptible person and infect them through direct faecal–oral transmission or the faeces may enter bodies of water. This can occur if people defecate directly into, or if sewage effluent has contact with water used for drinking. Animal carriers can also contaminate water supplies by defecating directly into surface water or if their faeces penetrate into groundwater that is either unconfined or that has a high water table. Soil type is also an important factor because it affects penetrability of pathogens into groundwater. Heavy rainfall events may play an important role as they may facilitate the spread of manure that contains the bacteria.

Once the bacteria enters water, it may remain there until it is ingested by a person as drinking water, during recreational activities, or using food as a vehicle. As microbiological studies have shown, *H. pylori* may have a limited survival time in water and thus colonization (i.e. successful transmission) may be dependent upon the amount of time between introduction of the bacteria in water and ingestion by a susceptible person. As temperature has been as an important variable with respect to survival in water, seasonal cycles may also be possible. Other factors, such as pH and the presence of specific microorganisms or suspended materials may also affect waterborne transmission. The absence of the bacteria in the original water source (i.e. river or well) may not prevent infection if it is delivered through a pipe or stored in a container where *H. pylori* exists in a biofilm. Treatment of water is probably helpful to minimize the risk of waterborne transmission. The Table outlines important components of the waterborne *H. pylori* transmission cycle.

Other possible transmission routes may involve direct person-to-person transmission through oral–oral routes [11, 90] and through direct exposure to animals.

This model incorporates many of the risk factors that have been identified for *H. pylori* transmission. For example, transmission via the waterborne pathway explains the higher prevalence and lower age of acquisition of *H. pylori* in developing countries. This is because the possibility for contamination of water is greater in areas where sewer systems and water treatment facilities are less developed. In addition, the time between *H. pylori* introduction into water and ingestion by a susceptible individual may be shorter because water treatment, a time-consuming and risk-reducing step, is less common. The absence of indoor plumbing, which is seen more often in the developing world, requires that people rely on the closest

available sources of water for drinking, regardless of contamination. A lack of running water also means that people bathe in open water, where ingestion may occur. The model also supports geographic clusters of infected individuals as the transmission cycle may be contained within susceptible water systems. Thus, the presence of different environmental influences, such as livestock, around water systems explains why infection is more common in certain areas. However, it is important to recognize that even if the most frequent route of transmission involves water, other routes are plausible and are probably responsible for at least a small proportion of cases as can be seen in the model.

CONCLUSION

The details of *H. pylori* transmission remain unclear, hampering efforts to reduce its burden of illness. Microbiological and epidemiological evidence have repeatedly indicated the possibility that water may be a reservoir in faecal–oral *H. pylori* transmission. The proposed model indicates that the risk of *H. pylori* infection is greater in areas where the presence of faecal matter in water intended for human consumption is more likely and explains why people depending on more susceptible water sources may be at higher risk of infection.

Future studies should employ a combination of methodologies from microbiology and epidemiology. As microbiological methods develop further, it may enhance our ability to understand the physical nature and important characteristics of the bacterium as it enters and colonizes the human stomach. Phenotyping and genotyping of strains isolated in humans and water will further clarify the steps involved in waterborne transmission. To date, many epidemiological studies investigating the relationship between drinking water and *H. pylori* infection have simply looked at water source but not the factors that may contribute to the presence of the bacterium in water. Researchers in future studies need to examine exposures to a number of known risk factors and others hypothesized to affect water quality including: water source (e.g. surface water and groundwater); type of groundwater source (e.g. confined, semi-confined, and unconfined); the presence of water treatment; type of sewage disposal system; surrounding land use (residential *vs.* agricultural and specific agricultural); and soil type around groundwater sources. By taking a closer look at these exposures,

water's role in *H. pylori* transmission may be clarified. If this is the case, it may enable the development of interventions that can reduce the burden of *H. pylori* disease in the population. In summary, this paper highlights important areas to address in future research, including investigation and specification of the mechanisms and processes by which water-related characteristics affect and influence the transmission of *H. pylori* infection.

DECLARATION OF INTEREST

None.

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