Helicobacter in water and waterborne routes of transmission

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1. SUMMARY

*Helicobacter pylori* is the causative agent of gastritis and duodenal ulcers and plays a role in the development of gastric cancer. The transmission of *H. pylori* remains unclear but two different pathways have been suggested: faecal–oral and oral–oral. Studies from developing countries with low socio-economic status and poor management of the drinking water suggest that environmental factors are important. Thus, the water may serve as a reservoir for *H. pylori* that infect humans. Viable but non-culturable bacteria are widespread in marine environments. The coccoid form of *H. pylori* in non-culturable by ordinary techniques and it remains to be studied whether this form is viable. Several studies have been performed to assess the viability of *H. pylori* in water since it has been suggested that the coccoid form of *H. pylori* is responsible for transmission in the environment, probably via contaminated water. Different DNA-based methods have been used to detect *Helicobacter* spp. in water. Target genes have been selected to avoid cross-reactivity between *H. pylori* and other bacteria. However, closely related bacteria and unknown subspecies of *Helicobacter* spp. may confound the ‘species-specific’ DNA-based assays and must be considered if controversial results appear in analyses of environmental samples.

2. INTRODUCTION

*Helicobacter pylori* colonizes the human stomach and establishes a chronic infection associated with an inflammatory response. *H. pylori* is the causative agent of gastritis and peptic ulcers and plays a role in the development of gastric cancer. However, only a subpopulation of infected individuals develop gastroduodenal disease. The prevalence of *H. pylori* infection in the world is assumed to be approximately 50%, with higher prevalence in developing than in developed countries. Genetic and socio-economic factors contribute to acquisition of infection. There appears to be no substantial reservoir of *H. pylori* aside from the human stomach (Dunn et al. 1997).

The epidemiology and transmission pathways of *H. pylori* infection are important for the understanding of this common worldwide infection (Taylor and Blaser 1991). The transmission of *H. pylori* remains unclear but two different pathways have been suggested: faecal–oral and oral–oral (Feldman et al. 1998). Different transmission routes may be predominant in different geographical areas. In developed countries, where sanitary procedures such as water treatment are well managed, reports show a clustering of *H. pylori* infection within families, which supports an oral–oral transmission pathway (Brenner et al. 1999). The source of *H. pylori* could be saliva and dental plaques since *H. pylori* organisms have been isolated from these locations (Ferguson et al. 1993; Nguyen et al. 1993). Studies from developing countries with low socio-economic status and poor management of drinking water suggest that environmental factors are more important than the oral–oral transmission route (Hopkins et al. 1993).

3. EPIDEMIOLOGICAL STUDIES

An association between the prevalence of *H. pylori* in Peruvian children and the source of drinking water has been shown (Klein et al. 1991). Transmission by the municipal water supply was suggested since children whose homes had external water sources were three times more likely to be infected with *H. pylori* than those whose homes had internal water sources. Hence, children living in homes with no

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internal plumbing had a higher level of infection than children living in homes with standard plumbing. In addition, children from high-income families with a municipal water supply had a 12-fold increase in the risk of being infected with *H. pylori* than children from high-income families with a water supply from community wells. It was concluded that socio-economic status in Peru was a less important risk factor than water source in acquiring the infection. In developed countries no association between *H. pylori* infection and water was evident (Hultén *et al.* 1998). Thus, the environment may serve as a reservoir for *H. pylori* that infect humans, at least in developing countries.

An increased risk for gastric cancer among sewage workers has been described in several studies (Lafleur and Vena 1991; Friis *et al.* 1993). These workers are often engaged at both municipal drinking water plants and sewage plants. Since *H. pylori* has been associated with an increased risk for cancer of the stomach, Friis *et al.* (1996) tested the hypothesis that sewage workers were at risk of infection by *H. pylori* in their work. The study design was cross-sectional and IgG antibodies against *H. pylori* were analyzed in 151 sewage workers and 138 referents (matched for age and socio-economic status). The previously described increase in the prevalence of IgG antibodies against *H. pylori* with increasing age was observed in both groups but the exposure to sewage work in Sweden did not cause an increased risk of infection with *H. pylori*.

Another study, which casts some doubts on the theory of faecal–oral transmission via a common source, was performed among foreign travellers (Lindkvist *et al.* 1995). This group studied seroconversion to *H. pylori* among 133 initially *H. pylori* seronegative young Swedes travelling to developing countries for a total of 16–48 years abroad. Serum samples were drawn before travelling. Gastroenteritis was considered as a marker of exposure to faecally contaminated food or water. Of the subjects, 66% reported one or more episodes of gastroenteritis. Not one of the 133 travellers coming for their second blood sample, drawn on average 4 months after returning to Sweden, seroconverted in the *H. pylori* ELISA. Thus, these two epidemiological studies could not provide any evidence for waterborne routes of *H. pylori* transmission.

### 4. VIABLE BUT NON-CULTURABLE *H. PYLORI* IN THE ENVIRONMENT

Viable but non-culturable bacteria such as *Salmonella* spp., *Campylobacter* spp., and *Vibrio* spp. are widespread in marine environments (Byrd *et al.* 1991). It has been suggested that the coccoid form of *H. pylori* is responsible for transmission in the environment, probably via contaminated water (Hultén *et al.* 1998). Possible mechanisms for the transmission of the coccoid form in the environment have been discussed. However, the coccoid form of *H. pylori* is non-culturable by ordinary techniques and it remains to be studied whether the coccoid form is viable. Several studies have been performed to assess the viability of *H. pylori* in water (West *et al.* 1992; Shahamat *et al.* 1993; Enroth *et al.* 1999). During the conversion from the rod-shaped into the coccoid form, numerous different morphological forms of *H. pylori* are evident (Bode *et al.* 1993) (Fig. 1). This transformation is a transient process that occurs during prolonged culture *in vitro*. The morphology is also affected by variations in the environment (oxygen stress, temperature and presence of antibiotics). Analyses show that the majority of coccoid bacteria have an intact cell wall, cell membrane and cytoplasm (Bode *et al.* 1993; Moshkowitz *et al.* 1994). Nutrient supplementation to coccoid forms has been tested but no conversion from coccoid to rod-shaped forms was observed (Sörberg *et al.* 1996). However, the addition of fresh medium somewhat increased the intracellular ATP-content (Sörberg *et al.* 1996). Changes in morphology, intracellular composition and surface properties in *H. pylori* during conversion from rod-shaped to coccoid forms have been studied (Enroth *et al.* 1999); rods have a higher density than cocci and bacteria stored in water have the lowest density. If the coccoid forms are viable yet smaller in size and volume, the density should theoretically be the same. The quantitative DNA, RNA and ATP content are reduced in coccoid bacteria and these forms also express fewer surface-related proteins. The degenerative changes found in this study point to dead bacteria and not to a viable but non-culturable form. However, it was not ascertained whether all coccoid forms have an overall low DNA, RNA and ATP content, or if a few cells are viable with full energy supplies and the remainder are empty, dead spheres. In addition, the response to stress such as starvation and ageing seems to be different from the response to acid stress as...
determined by assessing protein synthesis under various conditions (Mizoguchi et al. 1998). If coccoid *H. pylori* exhibits diversity in viability following exposure to different stresses, this may be of importance in the colonization process of the gastric mucosa. Only one report has shown a successful reversion of coccoid *H. pylori* to the rod shaped culturable form. This study was performed in mice but contradictory results have been reported in pigs (Eaton et al. 1995; Wang et al. 1995).

5. DETECTION OF ENVIRONMENTAL *H. PYLORI*

5.1. Culture

So far, no report has been published on successful culture of *H. pylori* from environmental samples. All bacillary *H. pylori* organisms change morphology gradually over 10 d of culture and enter a non-culturable stage after 7–10 d on an agar plate. It has been shown that such coccoid forms did not convert into the bacillary form during passage through eggs (Enroth and Engstrand 1996). Egg passage has been used for the isolation and growth of slow-growing organisms such as *Rickettsiae* spp. and *Chlamydia* spp., among others, and is a simple and rapid method of culturing and evaluating culturability of bacteria (Cox 1938; Fei Fan et al. 1957). It is, however, theoretically possible to grow the organism within a few days in water if the rod-shaped form is transferred to the water source. This is illustrated by patients with gastroenteritis and vomiting who may transfer the bacteria through a gastric–oral route (Parsonnet et al. 1999).

5.2. Microscopy

*H. pylori* was found in a majority of the surface and shallow groundwater samples examined in Pennsylvania and Ohio (Hegarty et al. 1999; see section 6). Fluorescent antibody staining was used with a *H. pylori*-specific monoclonal antibody with no cross-reactivity to closely related bacterial species. However, at least 24 species within the genus *Helicobacter* are described in the literature and there is a possibility of cross-reactivity among these *Helicobacter* species or other bacteria. The result in Hegarty’s study supports a waterborne route of transmission of the organism although no evidence of viability was shown.

5.3. Autoradiography

Findings based on an autoradiographic approach gave evidence supporting the hypothesis that there is a waterborne route of infection for *H. pylori* (Shahamat et al. 1993). Tritium-labelled cells of *H. pylori* revealed aggregations of silver grains associated with uptake by *H. pylori* of radio-labelled substrate. Temperature was a significant environmental factor associated with the viability of the organism in water. *H. pylori* remained viable as determined by autoradiography at temperatures of 4°C for 26 months. However, sterile water does not reflect the natural environment in which competition with natural populations of microorganisms can occur. The persistent coccoid form of *H. pylori* detected by autoradiography may play an important role in the transmission of disease.

5.4. DNA-based techniques

Different PCR methods have been used to detect *Helicobacter* spp. in environmental samples, gastric juice, faeces and gastric biopsy samples (Mapstone et al. 1993; Enroth and Engstrand 1995; Li et al. 1995). Target genes for PCR have been selected to avoid cross-reactivity between *H. pylori* and other bacteria. Such genes are the urease encoding gene *ureA*, the adhesin encoding gene *hpaA* and the cytotoxin associated geneA, *cagA*. Nonetheless, closely related bacteria and unknown subspecies of *Helicobacter* spp. could confound the ‘species-specific’ PCR assays and must be considered if controversial results appear in analyses of environmental samples. Several different animal species are infected by unique *Helicobacter* species and at least 24 named *Helicobacter* spp. as well as closely related unnamed bacteria have been reported so far. In addition, some features are shared between almost all *Helicobacter* spp. such as strong urease activity. Thus, the specificity of a DNA-based assay is not consistent and relies on the availability of up to date accurate sequence data. For detection of *H. pylori* organisms in stool and water samples, a pre-PCR step involving immunomagnetic separation (IMS) has been developed (Enroth and Engstrand 1995 Fig. 2). Rabbit hyperimmune antiserum was produced and magnetic beads were coated with purified IgG which bound to both coccoid and bacillary forms of *H. pylori*. Spiked faecal and water samples and a patient’s stool specimen all scored positive with the IMS-PCR technique.

6. PRESENCE OF HELICOBACTER SPECIES IN WATER

The IMS-PCR technique described in section 5.4 was used to provide an epidemiological link between *Helicobacter* spp. isolated from drinking water and the community (Hultén et al. 1996). Although non-specific amplification cannot be ruled out, the results were consistent with conclusions from the previous epidemiological study; for example, an indication for waterborne transmission of *Helicobacter* spp. in some environments was found. The study was performed in 1995 and several reports of closely
related bacteria and other *Helicobacter* spp. have been published since then.

Tap and well water and field soil samples were collected in a region of Japan with a high *H. pylori* infection rate and examined using a similar IMS-PCR technique to that described in section 5.4, but with a different target gene for the PCR reaction (Sasaki et al. 1999). Nucleotide sequencing of the nested PCR products was also performed. Although the authors observed that the conserved region in *ureA* is more specific than the target genes used in the Peruvian study (16S rRNA and *hpaA* adhesin gene), no further evidence of these conclusions has been published. The presence of *Helicobacter* spp. DNA in Swedish water was detected with the same primer set as that applied in the Peruvian study (Hultén et al. 1998). However, non-specificity of the PCR methods due to the presence of unknown or closely related bacteria could not be totally ruled out. This was illustrated when sequence alignment of the *H. pylori hpaA* gene showed high similarity to *H. nemestrinae* and *H. acinonyx* which indicates that subspecies of *Helicobacter* could confound the results. One possible explanation could be that surface water leaking into countryside household wells was contaminated with such subspecies.

The same problem must be considered in the study performed in the US (Hegarty et al. 1999). Although 10 *Helicobacter* subspecies were tested for cross-reactivity with the *H. pylori*-specific monoclonal antibody, it is impossible to state that the antibody is specific for *H. pylori* when there are at least seven known additional subspecies that have not been tested. In conclusion, future studies of routes of *H. pylori* infection must consider the possibility of controversial results due to the presence of unknown *Helicobacter* species in the environmental samples. The only *H. pylori*-specific assay is culture.

7. CONCLUSIONS

Evidence for waterborne transmission routes of *H. pylori* has been provided by a number of investigators. Despite the epidemiological evidence supporting a waterborne route of infection, there is little information available regarding the occurrence of the organism in groundwater, which is the main source of drinking water to private households. Detection of *H. pylori* in environmental samples is confounded by the lack of a standard procedure for the detection of this organism. Attempts to culture *H. pylori* directly from water samples have been unsuccessful, probably due to its conversion into a coccoid, non-culturable form. However, overgrowth of other microorganisms may provide false-negative results even if selective media is used. Other tools for detection of *H. pylori* in water, such as DNA-based techniques, microscopy using monoclonal antibodies and autoradiography, all have a potential for false-positive results.

*H. pylori* does not appear to exist in a culturable form outside the stomach and even though signs of *H. pylori* are detected in water or other environmental samples, there is still no evidence for transmission by this route. Future research in this field should focus on development of *H. pylori*-specific diagnostic tools such as identification of a *H. pylori*-specific gene as target a for PCR. Furthermore, attempts to optimize culture conditions for *H. pylori* in environmental samples could be one additional challenge. Studies of transmission pathways of *H. pylori* infection need to continue and are important for the understanding of this common infection. To prevent spread of *H. pylori*-associated gastroduodenal diseases, especially in the developing countries, we need an improved understanding of how *H. pylori* is transmitted between hosts and if there is any reservoir for the bacteria aside from the human stomach. Extended eradication strategies will lead to increased use of antibiotics in the community that will increase the risk of development and spread of resistance among *H. pylori* and other bacteria.

8. REFERENCES


