Occurrence of *Helicobacter pylori* in surface water in the United States

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J. P. Hegarty, M. T. Dowd and K. H. Baker. 1999. The primary mode of transmission of the human pathogen *Helicobacter pylori* is unresolved. This study examined the possibility that *H. pylori* is water-borne. Because methods for the direct culture of *H. pylori* from water samples remain elusive, a microscopic technique was used for detection of this organism. Actively respiring micro-organisms binding monoclonal anti-*H. pylori* antibody were found in the majority of surface and shallow groundwater samples tested (*n* = 62), indicating that *H. pylori* may be present in aquatic environments in the US and supporting a water-borne route of transmission for this organism. There was no significant correlation between the occurrence of either total coliforms or *Escherichia coli* in the water and the presence of *H. pylori*. Our results indicate that routine screening of water supplies for the presence of traditional indicator organisms may fail to protect the consumer from exposure to *H. pylori*.

**INTRODUCTION**

*Helicobacter pylori* is the principal cause of type B gastritis, peptic ulcer disease, gastric adenocarcinoma and MALT-lymphoma, and has been classified as a Class I carcinogen by the World Health Organisation (Blaser 1990; Aruin 1997). It is a virulent pathogen as evidenced by its low infective dose and high prevalence in human populations (Marshall 1991). Although *H. pylori* colonizes over 50% of the world’s population, no reservoir outside of the human stomach has been identified (Cave 1997; Dunn et al. 1997). Transmission presumably occurs through faecal–oral and oral–oral routes, yet the principal route of transmission remains unknown. Evidence favours a faecal–oral route (Xia and Talley 1997) and an epidemiological association between water sources and the prevalence of *H. pylori* infection has been reported by several researchers (Klein *et al.* 1991; Ramirez-Ramos *et al.* 1994; Mendall *et al.* 1992; Mitchell *et al.* 1992; Goodwin 1993; Hopkins *et al.* 1993; Goodman *et al.* 1996). Recently, *H. pylori* infection has been associated with the consumption of untreated well or spring water by children (Bunn *et al.* 1997; Carballo *et al.* 1997). Further evidence has been provided by culture of *H. pylori* from the faeces of infected individuals (Thomas *et al.* 1992; Kelly *et al.* 1994), maintenance of viability in a viable but non-culturable (VNC) form in water (West *et al.* 1992; Shahamat *et al.* 1993) and detection of *H. pylori*-specific nucleic acid sequences in water in Columbia, Peru and Sweden and sewage in the US (Handwerker *et al.* 1995; Hulten *et al.* 1996, 1998; Forrest *et al.* 1998).

Despite the epidemiological evidence supporting a water-borne route of infection for *H. pylori*, there is little information available regarding the occurrence of the organism in surface water and shallow groundwater. Shallow groundwater is frequently under the influence of surface water; rapid infiltration of surface water into shallow aquifers transfers surface water organisms, including bacteria, viruses, algae and protozoa, to these aquifers. Under these conditions, shallow groundwater may be considered a form of surface water. Untreated or minimally treated shallow groundwater is a frequent source of drinking water to private households in rural areas of the US (Yates 1997).

We have surveyed surface and shallow groundwater samples in Pennsylvania and Ohio, USA for the presence of *H. pylori*, total coliforms and *Escherichia coli*. The purpose of this study was twofold. Firstly, we analysed surface and shallow groundwater samples to determine the occurrence of *H. pylori* in untreated water in the US. Secondly, we evaluated the usefulness of traditional indicators of microbiological...
water quality (total coliforms and E. coli) in predicting the presence of H. pylori in these waters.

MATERIALS AND METHODS

Water samples from surface and shallow groundwater sources (n = 62) in Pennsylvania and Ohio were collected in Whirl-Pak Thio bags (Nasco, Fort Atkinson, WI, USA) and transported on ice to the laboratory for analysis. Samples were processed for the enumeration of H. pylori, total coliforms and E. coli within 24 h of collection. Of the samples examined, 42 were from surface water sources and 20 from shallow groundwater that served as an untreated source of drinking water. All of the shallow groundwater samples showed infiltration of surface water, as indicated by the presence of diatoms and other algal cells within the samples.

Actively respiring H. pylori were enumerated using combined fluorescent antibody-cyanoditoyl tetrazolium chloride (CTC) staining (Gribbon and Barer 1995; Pyle et al. 1995); CTC (2 mmol l\(^{-1}\) final concentration; Polyscience, Warrington, PA, USA) was added to a portion of the sample and the mixture incubated at 20 °C for 60 min in the dark. Cells were then fixed by the addition of formalin (final concentration 3.7%). After filtration of the aliquot (10–1000 ml depending on the amount of particulate matter in the water) onto a 0.2-μm pore polycarbonate black filter (Costar Scientific, Cambridge, MA, USA), the cells were stained using an indirect fluorescent antibody staining procedure. Monoclonal mouse anti-H. pylori antibody (Biodesign International, Kennebunk, ME, USA; IgG1; 6 μg ml\(^{-1}\) final concentration) was the primary antibody used. Data supplied by the manufacturer of the monoclonal antibody indicated that the antibody is specific for H. pylori and does not react with members of the Enterobacteriaceae. Additional testing in our laboratory demonstrated that the antibody does not react with H. pylori. The secondary antibody was goat anti-mouse IgG1 antibody (Biodesign International, Kennebunk, ME, USA; IgG1; 6 μg ml\(^{-1}\) final concentration) was the primary antibody used. Data supplied by the manufacturer of the monoclonal antibody indicated that the antibody is specific for H. pylori and does not react with members of the Enterobacteriaceae. Additional testing in our laboratory demonstrated that the antibody does not react with H. pylori. The secondary antibody was goat anti-mouse IgG1 antibody (Biodesign International, Kennebunk, ME, USA; IgG1; 6 μg ml\(^{-1}\) final concentration) was the primary antibody used. 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RESULTS

Figure 1 summarizes the detection of total coliforms, E. coli and H. pylori in the water samples examined. Actively respiring H. pylori were present in 25 of 42 (60%) of the surface water samples and 13 of 20 (65%) of the shallow groundwaters sampled in Pennsylvania and Ohio. Total coliforms were detected in 40 (95%) of the surface water samples and 14 (70%) of the groundwater samples, while E. coli was detected in 31 (74%) of the surface water samples and 9 (45%) of the ground water samples, respectively. The association between the presence of total coliforms or E. coli and H. pylori was evaluated using a χ² test (GraphPad Software, San Diego, CA, USA). No significant associations between the presence of either indicator organism and the presence of H. pylori could be detected (Table 1). In four samples from private

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**DISCUSSION**

*Helicobacter pylori* was found in the majority of the surface and shallow groundwater samples examined in Pennsylvania and Ohio. The detection of *H. pylori* in surface water and groundwater strengthens the argument for a possible waterborne route of transmission of this organism. Given the moderate number of samples examined and the restricted geographical area from which the samples were obtained, it is not possible to state that *H. pylori* is common in all aquatic habitats throughout the entire US. Additional studies are needed to thoroughly delineate the distribution of *H. pylori* in surface and groundwater.

**Table 1** Association between *Helicobacter pylori* and indicator organisms

<table>
<thead>
<tr>
<th>Total coliforms</th>
<th></th>
<th>Escherichia coli</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td><em>H. pylori</em></td>
<td>32</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>Positive</td>
<td>20</td>
<td>3</td>
<td>15</td>
</tr>
</tbody>
</table>

$\chi^2$ for total coliforms vs *H. pylori* = 0.002. $\chi^2$ for *E. coli* vs *H. pylori* = 0.057. Neither of these values is statistically significant ($P = 0.05$).

Enumeration of *H. pylori* in environmental samples is confounded by the lack of a standard procedure for the detection of this organism. Attempts to culture the organism directly from water samples (Hulten *et al.* 1996) have been unsuccessful due to the overgrowth of interfering organisms on the rich media used for this organism. In addition, *H. pylori* has been shown to enter a VNC state in water (West *et al.* 1992; Shahamat *et al.* 1993). Under these circumstances, the organism would not be recovered by traditional culture techniques. Both polymerase chain reaction and fluorescent antibody staining have been used successfully for the detection of *H. pylori* in clinical or environmental samples (Rivera *et al.* 1991; Rodewig *et al.* 1992; Husson *et al.* 1993; Sciortino 1993; Handwerker *et al.* 1995; Hulten *et al.* 1996, 1998). However, no accepted standard procedure for either of these methods currently exists and both have the potential for false-positive results. Thus, the results of any enumeration of *H. pylori* in environmental samples must be viewed as presumptive evidence.

The primary antibody used in this research was specific for *H. pylori*. It is important to note that absolute specificity of antibody preparations used in the examination of environmental samples cannot ever be unequivocally demonstrated. The possibility of cross-reactivity of the antibody with an organism other than *H. pylori* therefore must be considered. To control for possible cross-reactivity, the enumeration of *H. pylori* was performed in an extremely conservative manner. Only those cells that reacted with the antibody preparation and also had a size and shape consistent with *H. pylori* were counted. Given the morphological plasticity of *H. pylori*, which has been detected in both helical rod and coccoid forms, this approach may have resulted in an underestimation of the number of *H. pylori* present (Cellini *et al.* 1994). Negative controls (CTC and secondary antibody; no primary antibody) corrected for the possibility of counting either naturally fluorescing particles or micro-organisms capable of binding to the secondary antibody alone.

It must further be noted that, while CTC reduction is an indication of active respiratory enzymes, the ability to reduce CTC is not definitive for the presence of viable, infectious *H. pylori*. As Gribbon and Barer (1995) have noted, oxidative enzyme activities can be detected in non-culturable cells of *H. pylori*. Therefore, some of the CTC-reducing cells enumerated in the samples may not have been viable. Until a direct method to culture *H. pylori* from environmental samples is developed, however, the possibility that at least some of the cells detected were viable and infectious cannot be ruled out.

*Escherichia coli* was not detected in 50% (14 of 28) of the samples in which *H. pylori* was detected. The lack of a significant association between the presence of *E. coli* and *H. pylori* in the samples examined indicates that the use of *E. coli* for the determination of the potability of water may fail to protect individuals from infection with *H. pylori*. In
addition, the lack of association may indicate that *H. pylori* is able to survive longer in freshwater environments than *E. coli*, or even that *H. pylori* is part of the normal microbiota of many freshwater environments. Several studies have demonstrated that *H. pylori* can persist in a low nutrient environment similar to that of surface water (West et al. 1992; Shahamat et al. 1993).

Total coliforms were present in 85% (32 of 38) of the samples in which *H. pylori* was detected. While this observation hints at an association between *H. pylori* and total coliforms it is not conclusive. Samples evaluated in this research were collected from sites where faecal (human or animal) contamination was likely. Therefore, a high percentage of the samples were positive for the presence of total coliforms. Additional samples from pristine sites, where total coliforms are absent, are needed before an association between the occurrence of total coliforms and *H. pylori* can be confirmed.

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