

Presence of *Helicobacter pylori* in Drinking Water is Associated with Clinical Infection

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***Helicobacter pylori* was detected using molecular methods in untreated well water. The presence of *H. pylori* in the wells correlated with infection in consumers and with the presence of *Escherichia coli*, indicating fecal contamination. Consumption of untreated well water should be considered a risk factor for *H. pylori* infection.**

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INTRODUCTION

Although *Helicobacter pylori* colonizes >50% of the world's population, no reservoir outside of the human stomach has been identified. Transmission presumably occurs through fecal–oral and oral–oral routes. An epidemiological association between water sources and the prevalence of *H. pylori* infection has been identified (1–3). Further evidence for water as a vehicle for transmission has been provided by culture of *H. pylori* from the feces of infected individuals (4), maintenance of viability in water (5), amplification of *H. pylori*-specific nucleic acid sequences in water (6–8) and detection of actively respiring *H. pylori* in surface and groundwater (9). In the course of a survey of private untreated well water in Pennsylvania, USA, we found a significant association between the presence of *H. pylori* and clinical infection in individuals drinking the water.

MATERIALS AND METHODS

A total of 22 households (52 individuals) were involved in the study, all of whom had independently approached our laboratory because of either clinical symptoms (peptic ulcer disease, dyspepsia and/or gastric pain) or concerns regarding the microbiological quality of their drinking water. Diagnosis of *H. pylori* infection in individuals consuming the water was determined by the individual's private physician (using endoscopy, ¹³C-urea breath test and/or serology) and reported to our laboratory by the affected individual. It was not possible to obtain clinical data on all of the residents in the households. In these cases, individuals who were not tested for *H. pylori* were retrospectively assigned to a results group supporting the null hypothesis of no association between the presence of *H. pylori* in well water and clinical infection. Specifically, untested individuals drinking water from a well in which *H. pylori* was present were considered negative for *H. pylori* infection while untested individuals consuming water from wells negative for *H. pylori* were considered positive for infection.

Water samples (100–1000 ml) were obtained from a drinking water faucet within the house. Samples were collected after the surface of the faucet was disinfected with ethanol and the faucet flushed by turning the cold water tap on and allowing water to flow through the system for 3–5 min. Samples were collected in sterile 1 l plastic jars, placed on ice and returned to the laboratory

for analysis. Processing of samples was begun within 6 h of collection.

Water samples were analyzed for the presence of *Escherichia coli*, an indicator of sewage pollution, and for *H. pylori*. *E. coli* was detected using the membrane filtration technique with m-ColiBlue media (Hach, Loveland, CO). Actively respiring *H. pylori* was detected using combined fluorescent antibody–cyanoditoyl tetrazolium chloride (CTC) staining (9). For each sample analyzed, a control filter stained with CTC and secondary antibody only was also prepared and examined. This control allowed for the determination of non-specific fluorescence due to binding of the secondary antibody. Final counts for all samples were corrected for the negative controls.

H. pylori were identified on the basis of 3 criteria: intensity of the fluorescence; size; and shape. Cells that showed a bright (3–4 on a scale of 0–4) green fluorescence, were within the size range 1–3 µm and showed a curved-rod shape were considered to be *H. pylori*. Actively respiring *H. pylori* contained a fluorescent formazan crystal within the cell. Only those cells that reacted with the antibody and contained a reduced CTC–formazan crystal were counted.

E. coli and *H. pylori* were recorded on a presence/absence basis for each sample. Associations between presence of organisms were evaluated by contingency analysis using Graphpad Prism software (GraphPad Software, Inc., San Diego, CA).

Sufficient water was obtained from several of the households to allow for evaluation of the samples using a semi-nested PCR analysis. Oligonucleotide primers (Life Technologies, Rockville, MD) as previously described (10) were used for initial amplification of the ureA gene. A semi-nested upstream primer (5ATGAAGTGGGTATTGAAGCGATG3) was used to target a conserved, species-specific *H. pylori* methionine codon (ureA, amino acid 86) absent in all other sequenced *Helicobacter* spp. as determined by GenBank alignment. Water samples were concentrated onto 0.2-µm filters (Poretics) and genomic DNA was heat-extracted with Chelex-100 resin (Instagene, BioRad). Optimized 50 µl reactions contained 5 µl sample DNA, 10 mM Tris–HCl (pH 8.3), 75 mM KCl, 1.5 mM MgCl₂, 200 µM dNTPs, 0.3 µM primers and 1.5 U AmpliTaq Gold DNA polymerase (Perkin Elmer). A 10-min hot-start was followed by 30 cycles at 94°C for 30 s, 56°C for 20 s, 72°C for 30 s and a final extension at 72°C for 5 min. A 100-µl sample of the initial reaction was used as template for the semi-nested reaction for 20 cycles at an annealing temperature of 58°C. Under these reaction conditions, *H. pylori* generated a specific 203 bp amplicon that was separated by electrophoresis (5 V/cm, 90 min) in 2% agarose (3 : 1 NuSieve).

RESULTS

Table I summarizes the occurrence of *E. coli* and *H. pylori* in the well water samples from the households sampled and the associated clinical findings. Thirteen wells were positive for *H. pylori* by direct microscopic enumeration of cells stained using the combined fluorescent antibody-CTC (FACTC) technique. Both *E. coli* and *H. pylori* were found in 12 of these wells. Neither organism was found in 8 wells. *E. coli* was detected in 1 well in the absence of *H. pylori* and *H. pylori* was found in 1 well in which no *E. coli* was detected. There were statistically significant associations between the presence of *E. coli* and *H. pylori* in drinking water samples (Fisher's exact test, $p = 0.0011$) and between the presence of *H. pylori* in drinking water samples and clinical diagnosis of *H. pylori* infection (Fisher's exact test, $p = 0.0184$). At least 1 member of the household consuming the water from each of the wells in which *H. pylori* was detected was infected with *H. pylori*. This includes a household in which the sole resident, while asymptomatic for infection, was found to be colonized on clinical evaluation and a 3-member household in which all of the residents were colonized with *H. pylori*, 1 with symptomatic infection and 2 who were asymptomatic.

Water from 9 of the households was negative for *H. pylori*. In 4 of these households, individuals reported gastrointestinal symptoms. On clinical evaluation, only 1 individual was found to be *H. pylori*-positive while the remaining 3 individuals were diagnosed with gastroesophageal reflux disease.

Water samples from 5 households were screened for the presence of *H. pylori* using the semi-nested PCR procedure. Of these wells, 4 were positive for *H. pylori* and 1 was negative for the organism using the FACTC assay. PCR analysis independently confirmed the presence of *H. pylori* in the 4 wells that were positive using the FACTC assay. No *H. pylori* was detected using PCR in the 1 FACTC-negative well water sample tested.

DISCUSSION AND CONCLUSIONS

The present data demonstrate that actively respiring *H. pylori* can be detected in drinking water consumed in the US, and that consumption of such untreated drinking water is strongly associated with gastric colonization by this organism. These findings confirm previous observations made on samples obtained in Columbia (6), Peru (7) and Sweden (8) using PCR amplification of *Helicobacter* nucleic acid sequences.

The presence of *H. pylori* in private drinking water supplies was associated with the presence of *E. coli*, a traditional indicator of fecal pollution. In a prior study (9), we did not find a statistically significant association between *H. pylori* and indicator organisms (total coliforms or *E. coli*). The difference between these two studies, however, is easily reconciled. In our previous survey of surface- and groundwater (9), there appeared to be a general association between the presence of total coliforms and/or *E. coli* in a sample and the presence of *H. pylori*; however, there were

Table I. Summary of *H. pylori* monitoring and clinical data

Well No.	Drinking water		Number in household	Colonization with <i>H. pylori</i> ^a	
	<i>E. coli</i>	<i>H. pylori</i>		Positive	Negative
1	-	+	1	1	0
2	+	+	2	2	0
3	+	+	3	2	1 (1)
4	+	+	3	2	1 (1)
5	+	+	3	3	0
6	+	+	2	2	0
7	-	-	2	1 (1)	1
8	-	-	3	1 (1)	2
9	+	+	4	3	1 (1)
10	-	-	2	0	2
11	+	+	2	1	1
12	+	+	3	1	2 (2)
13	-	-	1	0	1
14	+	+	2	1	1
15	+	-	4	3 (2)	1
16	-	-	2	1 (1)	1
17	-	-	2	1	1
18	-	-	1	0	1
19	-	-	2	0	2
20	+	+	3	2	1 (1)
21	+	+	2	2	0
22	+	+	3	2	1 (1)

^aValues in parentheses indicate number of untested individuals assigned to a category.

not sufficient samples with no total coliforms or *E. coli* to allow for a statistical comparison to detect an association. In the present study, confined to groundwater samples from private drinking water wells, such an analysis is possible, and there is a significant association between the 2 organisms.

The most common water quality problem in rural water supplies is bacterial contamination from septic tank effluent (11). All of the households tested had on-lot septic tanks for the treatment of household sewage and wastewater. The presence of *E. coli* in the majority of the wells positive for *H. pylori* indicates that infiltration of domestic waste into these wells is the most likely source of the *H. pylori* detected. It is reasonable that, like *Campylobacter* spp., *H. pylori* do not significantly multiply outside their natural habitat, but may persist in the external environment, especially aquatic habitats. Under these circumstances, the *H. pylori* found in the wells may possibly be the result of contamination of the water by individuals in the household who are colonized with the organism rather than the source of infection to these individuals. As we do not have complete medical history data for each of the individuals involved in the study, we are unable to conclusively state that the source of infection to each individual was the drinking water evaluated. Regardless of the primary source of *H. pylori* in the individuals examined, the presence of *H. pylori* in well water could serve as a vehicle of transmission for *H. pylori* to other residents and/or a mechanism for reinfection of susceptible individuals after antibiotic therapy for the eradication of *H. pylori*.

An understanding of how *H. pylori* is effectively transmitted can allow for preventive infection control of this human pathogen. Infection prevention would be far more cost-effective than intensive antibiotic treatment of infected individuals. Our data indicate that physicians should inquire as to drinking water source in individuals being evaluated for *H. pylori* infection. For those individuals drinking untreated well water, the possibility of contaminated water should be considered as a risk factor. In addition, treatment of well water using standard methods such as ultraviolet irradiation or drip chlorination could be recommended as a means to prevent the spread of infection.

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