The association of drinking water quality and sewage disposal with *Helicobacter pylori* incidence in infants: the potential role of water-borne transmission

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ABSTRACT

The mode of transmission of *Helicobacter pylori*, a bacterium causing gastric cancer and peptic ulcer disease, is unknown although waterborne transmission is a likely pathway. This study investigated the hypothesis that access to treated water and a sanitary sewerage system reduces the *H. pylori* incidence rate, using data from 472 participants in a cohort study that followed children in Juarez, Mexico, and El Paso, Texas, from April 1998, with caretaker interviews and the urea breath test for detecting *H. pylori* infection at target intervals of six months from birth through 24 months of age. The unadjusted hazard ratio comparing bottled/vending machine water to a municipal water supply was 0.71 (95% confidence interval (CI): 0.50, 1.01) and comparing a municipal sewer connection to a septic tank or cesspool, 0.85 (95% CI: 0.60, 1.20). After adjustment for maternal education and country, the hazard ratios decreased slightly to 0.70 (95% confidence interval: 0.49, 1.00) and 0.77 (95% confidence interval: 0.50, 1.21), respectively. These results provide moderate support for potential waterborne transmission of *H. pylori*.

Key words | child, cohort studies, *Helicobacter pylori*, infant, infection, Mexican Americans, sewer, water

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INTRODUCTION

Infection with the bacterium *Helicobacter pylori* is a major cause of chronic gastritis and is strongly associated with increased frequency of peptic ulcer disease (NIH 1994; Nomura *et al.* 1994; Graham & Graham 1998) and gastric cancer (Nomura *et al.* 1991; Forman *et al.* 1994; Kuipers *et al.* 1995; Axon 2002). Although it is a worldwide health problem, prevalence is generally higher in developing countries and amongst the lower socioeconomic levels (Eurogast 1993; Parsonnet 1995; Pounder & Ng 1995; Torres *et al.* 2000). The diseases associated with *H. pylori* infection generally do not emerge until adulthood; reports from cohort studies, however, indicate that early childhood is the most likely time for acquiring this infection (Granstrom *et al.* 1997; Torres *et al.* 1998; Malaty *et al.* 2002).

Several modes of transmission have been suggested in the literature: person-to-person, waterborne, and zoonotic (Goodman & Correa 1995; Brown 2000). Much evidence supports person-to-person transmission, though other pathways have not been ruled out. Studies have found higher prevalence of infection among institutionalized populations (Vincent *et al.* 1994; Harris *et al.* 1995; Lambert *et al.* 1995; Bohmer *et al.* 1997) and other settings with crowded living conditions (Mendall *et al.* 1992; Mitchell *et al.* 1992*a*,*b*; Peach *et al.* 1997; Rothenbacher *et al.* 1998; Torres *et al.* 1998; Herbarth *et al.* 2001; Malaty *et al.* 2001; Moayyedi *et al.* 2002), as well as clustering of infection within families (Malaty *et al.* 1991; Goodman & Correa 2000). There is evidence in the literature to support several specific pathways for person-to-person transmission: fecal–oral (Thomas *et al.* 1992; Kelly *et al.* 1994; Sasaki *et al.* 1999) oral–oral (Lee *et al.* 1991; Megraud 1995; Allaker *et al.* 2002) or gastric–oral (from vomitus) (Axon 1997; Bohmer *et al.* 1997; Parsonnet *et al.* 1999). Pathways may vary with a country's stage of development: in developed countries or areas where sanitation is good, the oral–oral (or gastric–oral) may be the most frequent mode of transmission, whereas in developing countries or areas with a high level of diarrheal diseases and inadequate sewage treatment, the fecal–oral pathway may dominate.

The current study used data from a prospective bi-national cohort of infants residing along the U.S./Mexico border at El Paso, Texas and Juarez, Chihuahua, representing a transition zone between a developing and a developed economy. Specifically, the Juarez area in the north of Mexico has been ranked as having a 'medium high' level of development within the country, in contrast with a 'low' level of development in the southern region bordering Central America (Torres *et al.* 1998). Across the border, in contrast with most of the U.S., a higher percentage of the population of El Paso is living below the poverty level (13% vs. 24%, respectively) (Anonymous 2000*a*,*b*). The Hispanic population of El Paso County is 78% of the total (Anonymous 2000*a*), compared to 13% nationwide (Anonymous 2000*b*). A study using data from the Third National Health and Nutrition Examination Survey reported that U.S. Hispanics have a higher prevalence of *H. pylori* infection than the non-Hispanic white population (Staat *et al.* 1996). Based on demographic variation, it would be expected that *H. pylori* infection rates in El Paso would be higher than average in the U.S. and the rates in Juarez lower than expected for Mexico.

The U.S.-Mexico border community, therefore, provides a unique area for epidemiological research on the transmission of *H. pylori*. The specific intent of the current research was to investigate evidence of a waterborne transmission pathway for *H. pylori* infection by testing the hypothesis that access to clean water and a sanitary sewerage system reduces the rate of *H. pylori* infection in children.

METHODS

Design

This study is a prospective analysis of the effects of the source and treatment of drinking water and the availability of sewage disposal on the incidence of *H. pylori* infection in a cohort of young children living in the El Paso/Juarez U.S.-Mexico border region: the Pasitos Cohort Study. Information on the establishment of this cohort has been previously published (Goodman *et al.* 2003). The cohort was recruited from two neighboring communities lying on opposite sides of the border.

Infants were identified before birth by recruiting pregnant women in their last trimester who received services from Women, Infants & Children (WIC) clinics in El Paso County or maternal–child clinics operated by the Instituto Mexicano del Seguro Social (IMSS) in Juarez. Recruitment began April 1, 1998, and continued through October 31, 2000.

Targeted follow-up exams began at 6 months of age and continued every 6 months thereafter. Baseline demographic and exposure information was obtained by a structured questionnaire administered to mothers in person by interviewers before birth and follow-up exposure information was obtained at target intervals of six months. The current analysis utilized data from the first 24 months, covering target ages of 6, 12, 18 and 24 months, collected from October 1998 through November 2002.

Definition of infection status

Infection status was determined by the ^{13}C-urea breath test developed and subsequently modified by Graham and his coworkers (Graham *et al.* 1987; Klein & Graham 1993). After ingestion of ^{13}C-labelled urea in either fruit juice or water, the $^{13}C/^{12}C$ ratio in the collected breath samples was measured and compared to a baseline sample. The change over baseline (DOB) value was then used to estimate the urea hydrolysis rate (UHR). This quantity addresses one problem with evaluating a DOB value for children: they have a lower baseline carbon dioxide production rate than adults. Some researchers have argued that this translates into increased breath enrichment over baseline, resulting in false positives (Klein *et al.* 1999). The UHR corrects for the dependence of DOB on body size by correcting for body-size-dependent variations in CO_2 production, and is estimated from standard formulas that account for the age, sex, height and weight of each subject: UHR (μg/min) = CO_2 produced $*$ DOB $*$ 0.3463 (Klein *et al.* 1999; Nurgalieva 2004). The test was considered positive when the UHR value was greater than 10 ug/min.

Outcome variable

The disease frequency of interest was the incidence rate of the first detectable *H. pylori* infection in the study population. Children were assumed to be infection-free at birth, given evidence that perinatal transmission is unlikely (Goodman & Correa 1995). Incident cases were defined as positive ^{13}C-urea breath test results that occurred at the first follow-up exam or after a negative result; for children with more than one positive result, only the first was counted. Since *H. pylori* infection is generally asymptomatic, and the onset is not generally noticed, the onset of infection was assumed to have occurred at the midpoint of the interval between the last negative visit (or birth) and the first positive one. Person-months at risk were estimated as the age in months at the estimated time of infection onset. Children who were tested at the target age of 24 months and had no positive test results during the study period were considered to be negative for infection. For children who had no positive test results during the course of the study and who missed the visit at target age 24, person-months at risk was calculated as the age at the last visit. Some children who missed the 24-month visit attended a subsequent visit (beyond the follow-up period included in this analysis) and information on infection status and person-months at risk was updated at that time.

Exposure variables

Exposure variables investigated were source and quality of children's drinking water and method of sewage disposal. The questionnaires asked specifically about the source of the children's drinking water and how often this water was purified in the home. Water source was categorized as either municipal water or bottled/vending machine water, the latter category including any type of drinking water purchased in a bottle or from a vending machine. It was considered that the source was municipal when both municipal and bottled/vending machine water were reported as sources. A three-level source variable was also introduced: bottled/vending machine water, 'purified' municipal water vs. 'unpurified' municipal water. 'Purified' water was taken to mean water that was usually or always further treated in the home, with treatment methods including filtering, boiling and chlorine or iodine use. The sewage disposal variable was dichotomized as a connection to municipal sewer system vs. use of a septic tank or cesspool.

Potential confounders examined in this analysis included exposure variables related to sanitation and hygiene: source of bathing water, presence of a toilet, animal contact, swimming location, mother's frequency of hand washing with soap after diaper handling, water purification practices for older siblings prior to the cohort child's birth as a measure of overall hygiene, as well as various demographic and socioeconomic variables: gender, household crowding (persons/room), maternal education, income and country of residence. Category boundaries for crowding, income and maternal education were taken from a published study of the older siblings of the cohort children. (O'Rourke *et al.* 2003).

It was not possible to validate the mother-reported water exposure variables such as water purification, and hand-washing. This would have required extensive observation of subjects' water use behavior and the act of observation would likely influence the behavior such that it would not be known how well it matched normal practices. As far as the type of household water source, this information should be reasonably accurate since many interviews were conducted in subjects' homes and in cases when they were not, the interviewers were familiar with the water sources available in the areas where the subjects lived.

Statistical analysis

The proportional hazards regression model was used to estimate the effect of the exposure variables on the incidence rate; the hazard ratio was the resulting measure of association.

Unadjusted hazard ratios were estimated for water and sanitation variables as well as for selected demographic and socioeconomic status (SES) variables. Model-building was performed using SAS statistical software package procedures (Anonymous 2004). Variables chosen for the univariate analysis consisted of those most likely to be associated with transmission of infection, including hygiene factors, socioeconomic factors, gender and country. All were categorical variables and the criterion for inclusion in the final model was a p-value < 0.25 on the log-rank test of equality in time-to-event across categories. The variable for country was included in the model and used in product terms with all the other variables to identify effect-measure modification (statistical interaction). Interaction terms with p-values > 0.05 were not included in the final model (Anonymous 2004). The assumption of proportionality was tested by inserting time-dependent variables (interactions of the variable in question with time) into the final model. Models were then stratified by any time-dependent variables with p-values < 0.05. The estimated effects of the remaining variables in the stratified and unstratified models were then compared as a final test of proportionality. The proportional hazards model was fitted using the SAS PROC PHREG procedure (Version 9.0).

RESULTS

A comparison of demographic, socioeconomic and hygiene-related factors for the study population by country is presented in Table 1. Relative income levels in the two areas are comparable, though somewhat lower in the El Paso group; other SES-related variables (housing, crowding and maternal education) indicate that the Mexican group in this study is somewhat better 'housed', with a higher proportion in less crowded and family-owned homes, and with higher levels of maternal education on average than their El Paso County counterparts. The two groups are similar in access to municipal drinking water and availability of a flush toilet. On the other hand, 95% of the Mexican population had access to a public sewer compared with only 31% in the U.S., given that many El Paso County homes use private septic systems.

A summary of the *H. pylori* incidence data is found in Table 2. Of the 472 Pasitos Cohort children studied through 24 months of age, infection status data were available for 468. One hundred and twenty-eight had a detectable infection that was estimated to occur by 24 months of age; the mean estimated age at onset among those with a detectable infection was 11.4 months.

The unadjusted hazard ratios are shown in Table 3. Children who drank bottled or vending machine water had a 29% lower infection rate than children who drank municipal water (HR = 0.71, 95% CI: 0.50, 1.01). The combined categories of purifying municipal water or using bottled/vending machine water, compared to unpurified

Table 1 | Characteristics of the families of children 0–24 months (and gender of cohort child) tested for *Helicobacter pylori* in El Paso, Texas, and Juarez, Mexico, 1998–2002

Variable	Juarez No.	Juarez %	El Paso No.	El Paso %
Gender				
Boy	88	51.5	143	47.5
Housing				
Own	78	45.8	106	35.2
Rent	62	36.5	70	23.3
Other*	30	17.7	125	41.5
Crowding (†people/†rooms)				
≤ 1	127	74.3	113	37.5
> 1–2	43	25.2	169	56.2
> 2	1	0.6	19	6.3
Animal contact‡				
Yes	93	54.4	139	46.2
River/pool bathing/ swimming				
Yes	163	95.3	255	85.0
Mother's education				
≤ 6 years	44	25.7	32	10.6
> 6 < 12	94	55.0	118	39.2
≥ 12	33	19.3	151	50.2
Household income§	$N = 151$		$n = 263$	
Very low	39	25.8	83	31.6
Low	95	62.9	159	60.5
Moderate	17	11.3	21	8.0
Source of child's drinking water				
Municipal, indoor tap	79	46.2	111	36.9
Municipal, outdoor tap	16	9.4	2	0.7
Bottled	75	43.9	160	53.2
Vending machine	1	0.6	27	9.0
Well	0		1	0.03
Purification of child's drinking water‖	$N = 95$		$n = 109$	
Always	73	76.8	70	64.2
Usually	8	8.4	4	1.1
Sometimes	3	3.2	6	5.5
Not usually	0	0	1	0.9
Never	11	11.6	28	25.7
Household drinking water source	$N = 170$		$n = 301$	
Municipal, indoor tap	101	59.4	211	70.1
Municipal, outdoor tap	6	3.5	1	0.3
Bottled	57	33.5	58	193
Vending machine	1	0.6	28	9
Public tap	3	1.8	3	1.0
Tanker	2	1.2	0	
Water purification (older sibling)	$N = 86$		$n = 164$	
Always	24	27.9	25	15.2
Usually	3	3.5	3	1.8
Sometimes	7	8.1	8	4.9
Not usually	3	3.5	4	2.3
Never	49	57.0	124	72.1
Source of child's bathing water				
Municipal, indoor tap	143	83.6	291	96.7
Municipal, outdoor tap	25	14.6	1	0.3
Well	0	0	1	0.3
Public tap	0	0	2	0.7
Tanker truck	3	1.8	1	0.3
Other	0	0	4	1.3
Sewage disposal				
Sewer	162	94.7	94	31.2
Septic tank	4	2.3	200	66.5
Cesspool	5	2.9	7	2.3
Latrine/toilet				
Latrine	8	4.7	5	1.7
Flush toilet	162	95.3	296	98.3
Mother's hand washing¶				
Always	107	62.9	226	75.1
Usually	34	19.9	45	15.0
Sometimes	23	13.5	24	8.0
Not usually	6	3.5	3	1.0
Never	1	0.6	3	1.0

*Live with relative/friend.

†Respondents were asked: "How often do you purify the children's drinking water?".

‡Pet ownership or the presence of farm animals.

§Income levels: (O'Rourke *et al.* 2003). Juarez: very low = <2,000-pesos/month; low = 2 − <6,000 moderate 6,000 > 9,000. US: very low < $10000/yr; low = 10 − <25,000; moderate 25 < 50,000.

‖Limited to those who used municipal water, well water or public tap water.

¶Respondents were asked "How often do you use soap (or a disinfectant) to wash hands after changing a dirty diaper?".

Table 2 | Incidence rates of *Helicobacter pylori* and attendance information for children 0–24 months in El Paso, Texas, and Juarez, Mexico, 1998–2002

	Total	Juárez	El Paso
N	472	171	301
Outcome data available	468	170	298
Cases	128	45	83
Person months	7,741	2,796	4,945
Rate/mo	0.0165	0.0161	0.0168
Rate/yr	0.198	0.193	0.201
Avg. months to infection among those infected within the 24 month period	11.4	11.3	11.5

municipal water, was associated with only a 10% decrease in the infection rate (HR = 0.90, 95% CI: 0.53, 1.52). With a three-category drinking water variable: home treatment of municipal water was minimally associated with a decrease in the rate of infection (HR = 0.97, 95% CI: 0.55, 1.70) compared to drinking municipal water that was not treated in the home. However, drinking bottled/vending machine water, compared to untreated municipal water, was associated with a 31% decrease in the rate (HR = 0.69, 95% CI: 0.40–1.20). Also showing a relatively low infection rate were cohort children whose caretakers reported at baseline (i.e., prior to their birth) that they treated municipal drinking water for the children in the household compared to those whose caretakers reported at baseline that they did not treat municipal drinking water for children in the household (H.R. = 0.54, 95% CI: 0.34–0.86.).

A 15% reduction in the rate of infection was observed for the contrast between having a connection to a sewer line and other types of sewage disposal (HR = 0.85, 95% CI: 0.60, 1.20). Among the covariates, fewer years of maternal education was associated with increased rates of infection; compared to at least 12 years of education, HR = 1.80 (95% CI: 1.10, 2.93) for 6 years of education or less and HR = 1.31 (95% CI: 0.88, 1.95) for 7–11 years of education. Country of residence was not associated with infection rates.

The adjusted hazard ratios are presented in Table 4. In addition to the exposure variables of water source and sewage disposal, the model also includes maternal education as the only covariate to meet the variable selection criteria. The country variable, while not meeting selection criteria, was retained to assess country-specific hazard ratios.

The adjusted hazard ratio associated with drinking bottled or vending machine water was 0.70 (95% CI; 0.49–1.00) and indicates that the incidence rate in children who drank bottled or vending machine water was 30% lower than the rate in children who had a municipal source of drinking water. After adjustment, there was a weaker decrease in the infection rate associated with having a connection to a sewer line vs. using a septic tank/cesspool.

No interaction was detected between the variable for country and the indicator variables for water, sewage disposal and education. The tests of proportionality resulted in p-values < 0.05 for the sewage disposal and education variables suggesting that these two variables might not be proportional over the time period (data not shown). The model was stratified by sewerage or education; with no change in model estimates. Thus, the stratified models are not presented.

A second model estimated the effect of treatment of municipal water in the home by comparing the combination of bottled/vending machine water and municipal water that was subsequently home-treated vs. municipal water that was not subsequently treated. No effect of home treatment of municipal water was apparent using this contrast: H.R = 1.00 (95% CI: 0.57–1.78).

DISCUSSION

Source of drinking water

It has been difficult to obtain conclusive evidence that confirms or rules out waterborne transmission of *H. pylori*. A few studies have reported the detection of *H. pylori* DNA in water samples, (Enroth & Engstrand 1995; Hulten *et al.* 1996; Hulten *et al.* 1998; Theron & Cloete 2002) but it is unclear from DNA evidence whether the detected organisms are viable for transmission. There is only one published report of the culturing of *H. pylori* from an environmental water sample: wastewater in Mexico (Lu *et al.* 2002).

There are limited data on the association of waterborne transmission indicators and *H. pylori* incidence. Most of the

Table 3 | Unadjusted hazard ratios as estimates of the effect of indicator variables on the incidence rate of *Helicobacter pylori* infection from a Cox proportional hazards model among children 0–24 months of age tested in Juarez, Mexico, and El Paso, Texas, 1998–2002

Variable	Unadjusted HR (95% CI)*	*p*-value
Drinking water source (cohort)		
Municipal source	1.0	
Bottled/vending machine	0.71 (0.50,1.01)	0.0523
Drinking water quality (cohort)		
Unpurified†	1.0	0.6975
Purified‡	0.90 (0.53,1.52)	
Source/quality (cohort)		
Municipal, not usually purified	1.00	
Municipal, usually purified	0.97 (0.55,1.70)	0.9254
Bottled/vending machine	0.69 (0.40,1.20)	0.1875
Source/quality (siblings)		
Municipal, not usually purified	1.00	
Municipal, usually purified	0.54 (0.34,0.86)	0.0094
Bottled/vending machine	0.75 (0.50,1.13)	0.1682
Sewage disposal		
Septic tank/cesspool	1.0	
Sewer connection	0.85 (0.60,1.20)	0.3603
Bathing water source (children)		
Municipal source	1.0	
Other	0.90 (0.29,2.83)	0.8529
Toilet facilities		
Latrine	1.0	
Flush toilet	1.95 (0.48,7.87)	0.3504
Country		
US	1.0	
Mexico	0.94 (0.66,1.36)	0.7702
Gender		
Male	1.0	
Female	1.13 (0.80,1.60)	0.5026
Maternal education		
≥ 12 years	1.0	
> 6 < 12	1.31 (0.88,1.95)	0.1785
≤ 6	1.80 (1.10,2.93)	0.1098
Income		
Moderate	1.0	
Low	1.88 (0.87,4.08)	0.1074
Very low	1.31 (0.57,3.00)	0.5204
Crowding§		
≤ 1/room	1.0	
> 1/room	0.87 (0.61,1.23)	0.4159
River/pool bathing/swimming		
No	1.0	
Yes	0.78 (0.43,1.42)	0.4183
Animal contact		
No	1.0	
Yes	0.86 (0.61,1.22)	0.4051
Hand washing		
Always	1.0	
Not always	1.00 (0.69,1.46)	0.9695

*HR, hazard ratio; CI, confidence interval.
†Purified water refers to municipal water that is always or usually treated in the home or bottled/vending machine water.
‡Unpurifed water refers to municipal water that is not always or usually treated in the home.
§Crowding was dichotomized for this analysis because of the small number of subjects with a crowding value > 2.

epidemiological data on water source and infection is from cross-sectional studies. Incidence data in the literature are scarce, particularly for young children. To date, only three cohort studies have been published that specifically investigated the risk of infection associated with water source in the very young (Lindkvist *et al.* 1999; Naficy *et al.* 2000; Sinha *et al.* 2004). An Egyptian study followed 155 children under 3 years old for 4 months, and reported an OR = 1.0 (95% CI: 0.15, 4.7) when comparing infection frequencies in children with a non-municipal source to those with municipal water (Naficy *et al.* 2000). The second study followed 121 2-to-4-year-old Ethiopian children for 30 months (Lindkvist *et al.* 1999). The investigators found that drinking water sources was a predictor of infection, when comparing water from a well to water from either a river or pipes: (RR = 1.46; 95% CI 1.0, 2.15), although no adjustment was made for possible confounding by SES or sanitation factors other than 'mother still chewing food'. And a study of 50 children in a Canadian First Nations community (1–13 years old) followed for 1 year (Sinha *et al.* 2004) revealed the presence of *H. pylori* DNA in 1 of 11 water samples from infected households compared to none

Table 4 | Adjusted hazard ratios as estimates of the effect of indicator variables on the incidence rate of *Helicobacter pylori* infection obtained from a Cox proportional hazards model among children 0–24 months of age tested in Juarez, Mexico, and in El Paso, Texas, 1998–2002

Variable	HR (95% CI)*
Drinking water source	
Municipal source	1.0
Bottled/vending machine	0.70 (0.49,1.00)
Sewage disposal	
Septic tank/cesspool	1.0
Sewer connection	0.77 (0.50,1.21)
Maternal education	
≥ 12 years	1.0
> 6 < 12	1.35 (0.90–2.03)
≤ 6	1.91 (1.14–3.21)
Country	
US	1.0
Mexico	0.90 (0.56,1.45)

*HR, hazard ratio; CI, confidence interval.

of the 12 samples from uninfected households. The authors commented that the number of organisms in the water may have been below some critical level for observation, but speculated, nevertheless, that water is a 'possible, but unlikely mode' of transmission in their study population.

There is little consistency among the additional published reports on the association between source of drinking water and *H. pylori* infection. A German study found an increased prevalence of *H. pylori* infection associated with a non-municipal water source compared to a municipal source in school-aged children (OR, 16; 95% CI:, 3.1, 89) (Herbarth *et al.* 2001). A study from Colombia reported that drinking stream water (as opposed to tap or well water) was associated with a higher prevalence of infection (OR 2.8; 95% CI: 1.2, 6.8) (Goodman *et al.* 1996). Other papers that specifically mention the effect of water source on *H. pylori* frequency reported null or minimal associations. A study from Taiwan (Teh *et al.* 1994) reported an OR = 1.2 (95% CI: 0.8, 1.7) when comparing seropositivity among children who drank well water to seropositivity among those who had access to tap water. A cohort study from Egypt estimated an OR = 1.0 (95% CI: 0.15, 4.7) comparing 'other' drinking water sources to a municipal source (Naficy *et al.* 2000).

The inconsistencies in the results across studies may be explained by several factors including the age range of the subjects. Since infection occurs most frequently in early childhood (Torres *et al.* 2000), evaluating risk factors in older age groups may be irrelevant, unless it is assumed that the exposure has not changed since childhood. Several reports included individuals from infancy through old age, but did not report the proportion of the study population that was young children (e.g., ≤age 5 years) (Mitchell *et al.* 1992*a*,*b*; Teh *et al.* 1994). Other studies focused on children and teenagers (≤20 years), but again provided no details on the distribution of age groups (Klein *et al.* 1991; Begue *et al.* 1998; Olmos *et al.* 2000).

In studies of waterborne transmission, the definition of exposure should include not just source of drinking water, but potability. A municipal source of drinking water is not necessarily a source of potable drinking water and protection from exposure to feces in drinking water cannot be assumed simply because a population has access to a piped municipal water supply. *H. pylori* DNA was found in municipal water in both Peru (Hulten *et al.* 1996) and in Sweden (Hulten *et al.* 1998) and in a water main in Scotland (Park *et al.* 2001). A median residual chlorine level of 1.1 mg/liter is reported sufficient to inactivate *H. pylori* (Johnson *et al.* 1997); Mexico City drinking water has residual chlorine levels at 0.93 mg/liter (Mazari-Hiriart *et al.* 2001), which may not totally eradicate the bacteria. On the other hand, a study in Mexico (Mazari-Hiriart *et al.* 2001) did not detect *H. pylori* in treated water. In any case, potability of drinking water, rather than source alone, gets closer to the actual exposure of interest: whether or not the children being compared drank clean or contaminated water. The current study considered the source and potability of water in a cohort design that followed children from birth to 2 years of age, the period when the initial acquisition of *H. pylori* infection frequently occurs.

The infection rate among children who drank bottled or vending machine water was reduced when compared to the rate among children who used a municipal source of water. However, the fact that treatment of municipal water in the home was not clearly associated with a reduced incidence rate indicates the challenges in evaluating effects of water purification. Alternate explanations for these results are 1) municipal water consumed in this cohort was generally

pathogen-free whether treated in the home or not, and the consumption of bottled/vending machine water was a marker for wealth rather than hygiene; 2) water purification practices may be subject to misclassification as people may be likely to overestimate how often they purify water; and 3) purification of municipal water may not be uniformly effective due to variation in technique. Investigators working in this area have found a wide range of home water treatment practices ranging from filtering with a piece of cloth, to manual chlorination, to the use of carbon filters on refrigerated pitchers (J. VanDerslice, personal communication).

Access to sanitary sewerage

It has also been difficult to confirm or rule out a role of excreta disposal in the transmission of *H. pylori*. Bacterial DNA has been isolated from feces (Thomas *et al.* 1992; Sasaki *et al.* 1999) and a few investigators have reported culturing viable organisms from feces (Thomas *et al.* 1992; Kelly *et al.* 1994; Parsonnet *et al.* 1999). As with water samples, however, attempts at culturing *H. pylori* from feces have been largely unsuccessful.

Effects on *H. pylori* associated with the frequency of access to a sanitary sewerage system has been evaluated in several studies (Oliveira *et al.* 1994; Katz *et al.* 1997; Souto *et al.* 1998; Redlinger *et al.* 1999; Olmos *et al.* 2000). These studies have reported inconclusive or minimal differences in prevalence of infection between children with and without access to a sewer network. In this study the rate of infection was somewhat reduced when there was access to a sewerage system compared to other forms of sewage disposal (primarily septic tanks) although the HR was weak and imprecise. This may reflect the fact that well-designed septic tanks pose minimal risk of exposure, while the sewer system in some parts of Cuidad Juarez includes open channels where there is the risk of direct contact with sewage.

Control variables

In the current study a lower level of maternal education was associated with an increased incidence rate. With respect to the other control variable, country of residence, incidence rates were slightly higher in the U.S. compared to Mexico and the unadjusted hazard ratio suggests that living in Mexico was protective. As noted above, the El Paso children in this study are of lower SES than average for the U.S. and the Juarez children are of higher SES than average for Mexico, thus the differences between children separated by the border are less than the average differences between their respective countries.

There is reason to believe that the estimates of effect for water source and sewage disposal may be biased towards the null due to at least two sources of bias. The number of infections was probably underestimated because there was no way to detect transient infections that were acquired and cleared between follow-up visits; as shown in a previous analysis of data from this cohort (Goodman *et al.* 2005), the infection rate was higher in children with more frequent follow-up, suggesting that the probability of detecting infections was associated with the length of follow-up intervals and therefore a notable number of infections were likely missed, particularly in children with less frequent follow-up. Misclassification of infection status is also likely as the breath test is not well-validated in infants and toddlers. If underestimation of infection frequency or misclassification of infection status is non-differential with respect to exposure status, and there is no evidence to suggest that it is not, the resulting effect estimate will be biased towards the null, provided that the misclassification is independent of other errors. Additionally, water source (either bottled/vending machine water or municipal water) may be an inadequate surrogate for the relevant exposure, and the results, therefore, may not reflect the association of infection rates with *H. pylori*-contaminated water. Municipal water on both sides of the border may be sufficiently treated such that the incidence rate associated with water presumed to be of higher quality (drinking bottled/vending machine water) would not be appreciably different from the incidence rate associated with drinking municipal water, resulting in a hazard ratio that underestimates the effect of contaminated water.

A limitation of the study is that the case definition relies on a diagnostic test of uncertain accuracy in the age group under study. Although validation studies of the urea breath test have shown excellent accuracy in school-aged children, there are questions about its accuracy in infants and toddlers (Torres *et al.* 2000; Goodman *et al.* 2003;

Nurgalieva 2004). Another limitation is the lack of information on the timing of infection onsets. The interpolation of age at infection from the data is an imperfect method. If infection occurs on average 'earlier' rather than 'later' in an interval, using the interval midpoint will result in an overestimate of the average age at infection and bias the estimated incidence rates by overestimating the person-months-at-risk and thus underestimating the incidence rate. Both of these sources of error, however, would be likely to underestimate exposure effects rather than produce falsely positive associations. Another limitation is not knowing the source and quality of the "bottled/vending machine" water.

In conclusion, this study reports prospective data that moderately support the hypothesis of waterborne transmission of *H. pylori*: both access to purified water and a sanitary sewerage system were moderately associated with a decreased rate of infection. Considering that potability of drinking water may be a more relevant exposure variable than source alone, it would be worthwhile in future studies to include information on water treatment in the home.

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