Fecal and Oral Shedding of *Helicobacter pylori* From Healthy Infected Adults

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Context *Helicobacter pylori* commonly infects humans; however, its mode of transmission remains unknown.

Objective To determine how humans—the primary host for *H pylori*—shed the organism into the environment.

Design Controlled clinical experimental study conducted from February through December 1998.

Setting Clinical research unit of a hospital in northern California.

Patients Sixteen asymptomatic *H pylori*–infected and 10 uninfected adults.

Intervention A cathartic (sodium phosphate) and an emetic (ipecac) were given to all infected subjects and an emetic was given to 1 uninfected subject.

Main Outcome Measure Confirmed *H pylori* isolates cultured from stool, air, or saliva before and after catharsis and emesis and from vomitus during emesis. Isolates were fingerprinted using repetitive extragenic palindromic (REP) polymerase chain reaction and species identity was confirmed by sequencing the 16s ribosomal RNA gene.

Results All vomitus samples from infected subjects grew *H pylori*, often in high quantities. Air sampled during vomiting grew *H pylori* from 6 (37.5%) of the 16 subjects. Saliva before and after emesis grew low quantities of *H pylori* in 3 (18.8%) and 9 (56.3%) subjects, respectively. No normal stools and only 22 (21.8%) of 101 induced stools grew the organism, although 7 (50.0%) of 14 subjects had at least 1 positive culture (2 stool culture samples were contaminated by fungus and were not included). Fingerprints of isolates within subjects were identical to one another but differed among subjects. No samples from uninfected subjects yielded *H pylori*.

Conclusions *Helicobacter pylori* can be cultivated uniformly from vomitus and, occasionally, from saliva and cathartic stools. The organism is potentially transmissible during episodes of gastrointestinal tract illness, particularly with vomiting.

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HELICOBACTER PYLORI CAUSES peptic ulcers and has been implicated in the etiology of distal gastric cancer.[1] However, it is not known how *H pylori* is transmitted. This uncertainty stems from 4 unresolved questions: (1) how does the organism leave its host and enter the environment? (2) where in the environment does the organism reside? (3) when do people acquire infection? and (4) are all people susceptible to infection? While most epidemiological evidence supports direct person-to-person transmission, the manner in which this occurs is unknown.[2-4]

Helicobacter pylori is thought to reside normally only in the stomach; thus, the organism is presumed to enter the environment in feces, saliva, or vomitus. *Helicobacter pylori* is a relatively fastidious organism, however, making its identification in clinical specimens difficult. Experienced laboratories may recover *H pylori* from only 50% to 70% of infected gastric biopsies.[5,6] From stool, saliva, and vomitus—which can be heavily colonized by more robust organisms—recovery of *H pylori* is even more difficult.[7-10] Thus, many clinical studies have relied on polymerase chain reaction (PCR) for *H pylori* identification.[11,12] Unfortunately, PCR cannot distinguish between DNA from viable cells and nonviable organisms. A new PCR-based method, immunomagnetic separation (IMS) with PCR, may remedy this problem by preferentially amplifying DNA within intact cells.[13-15]

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In this study, using both culture and IMS PCR, we evaluated whether *H pylori* could be recovered from feces, vomitus, and saliva of asymptomatic, infected adult volunteers. Since some studies suggest that *H pylori* is excreted only in diarrheal stools, we cultured stools both before and after administration of a cathartic. In addition, we sampled the air during episodes of vomiting. In this manner, we hoped to elucidate how *H pylori* enters the environment to invade new hosts.

METHODS

Subjects

We recruited healthy volunteers by advertising on radio stations and in business establishments, clinics, and churches. We preferentially recruited in minority (black and Hispanic) populations known to have high prevalence of *H pylori* in northern California.[16] During 3 weeks of announcements, we received 379 inquiries and interviewed 132 potential participants; 103 were eligible

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to participate. Exclusion criteria included age older than 55 years; pregnancy; history of ulcer disease, gastrointestinal tract bleeding, or severe dyspepsia; routine use of cathartics; recent use of antibiotics, histamine antagonists, or proton pump inhibitors; and prior treatment for *H pylori* infection.

Of the 103 eligible subjects, 62 subjects elected to participate. After providing written informed consent, each subject contributed a serum sample for *H pylori* IgG. The first 15 of the 27 seropositive subjects were invited to undergo 13C breath testing for *H pylori* (Meretek Corp, Nashville, Tenn), physical examination, blood cell counts, blood chemistries, and stool occult blood testing. Two asymptomatic subjects identified as *H pylori* IgG positive in a previous study asked to participate and were also invited for breath testing and physical examination. All 17 seropositive subjects were confirmed to be infected with *H pylori* by breath test but 1 was excluded from further participation because of anemia. The remaining 16 subjects (the *H pylori*–infected group) were invited to undergo the clinical experiment described herein. Ten *H pylori* serology– and breath test–negative volunteers were identified (the *H pylori*–uninfected group). Subjects were paid for their participation in the study.

Clinical Experiment

Helicobacter pylori–infected subjects were admitted to the general clinical research unit, where they were administered 45 mL of sodium phosphate solution in 90 mL of water, followed by 720 mL of water. We chose sodium phosphate as the cathartic because it has a rapid time of onset, acts on both the small and large bowels, and has been used previously to facilitate diagnosis of gastrointestinal tract pathogens.[17] We collected all stools during the 8 hours following cathartic administration and immediately transported them to the laboratory.

After an overnight fast, infected subjects were administered 5 mL of ipecac followed by at least 480 mL of water. Prior to emesis, we obtained a saliva sample (the subject spit into a cup) and placed a Mattson-Gavin air sampler 0.3 m away from the subject. For the duration of the emesis period, we sampled air onto sheep blood trypticase soy agar plates with a fluoropore filter centrally covering half the plate's diameter. We replaced the plate every 30 minutes to prevent desiccation. For 10 subjects, we placed a second air sampler 1.2 m away to determine the radius of bacterial aerosolization. Samples were transported to the laboratory for processing within 10 minutes of emesis. After vomiting had subsided, we collected a second sample of saliva.

The 10 uninfected control subjects provided normal stool and saliva samples for analysis. One uninfected control also underwent the emesis portion of the experiment.

The protocol was approved by the Stanford University Administrative Panel on Human Subjects in Medical Research.

Cultures of Stools, Vomitus, and Saliva

We diluted stool samples to a 20% suspension in phosphate-buffered saline (PBS) and sieved the suspension through a 250-µm strainer. We plated a 200-µL portion of the suspension on sheep blood trypticase soy agar supplemented with polymyxin B (3.3 µg/mL), amphotericin B (50 µg/mL), bacitracin (200 µg/mL), nalidixic acid (10.7 µg/mL), and vancomycin (100 µg/mL). We cultured a second portion (1 mL) of the suspension using the method described by Kelly and colleagues.[8] Using these methods, we successfully recovered *H pylori* from inoculated stools at concentrations as low as 10^2 organisms/mL (American Type Culture Collection strain 43579).[14] Sensitivity of *H pylori* detection varied, however, depending on the strain of *H pylori* inoculated (range, 10^2-10^4 organisms/mL).

We neutralized vomitus specimens to a pH of 7.0 and diluted the specimen with PBS at a 1:1 ratio; 200 µL was cultured on antibiotic plates as described herein. Using these methods, we were able to detect *H pylori* inoculated into vomitus at concentrations as low as 10 organisms/mL, depending on the strain of *H pylori* used.[14] Ipecac (0.125 mL per milliliter of sample) decreased sensitivity of *H pylori* detection 10-fold. Saliva samples were diluted 1:1 in PBS and plated as described.

All plates were microaerophilically incubated at 37°C. Suspicious colonies were confirmed as *H pylori*. When possible, we estimated the number of colony-forming units (CFUs) of *H pylori* per milliliter of sample.

IMS With Culture and PCR

We bound purified polyclonal rabbit anti–*H pylori* IgG (Dako, Glostrup, Denmark) to magnetized polystyrene beads precoated with sheep anti–rabbit IgG as described by the manufacturer (Dynal, Oslo, Norway). We then mixed the stool, vomitus, and saliva suspensions with 30 µL (1.8×10^6 beads) of the coated beads for 1 hour at 4°C. We separated the beads from the solution using a magnetic particle concentrator, discarded the solution, and resuspended the beads in 1 mL of PBS with 0.1% bovine serum albumin. After 3 such separations and washings, 1 portion of the separated bead-bacteria complex was resuspended in 30 µL of sterile distilled water, boiled for 10 minutes to lyse the bacteria, briefly chilled on ice, and frozen until analyzed by PCR. A second portion of the bead bacteria complex was resuspended in 100 µL of PBS and cultured as described herein. Because we found that IMS did not improve culture sensitivity, we did not culture the bead-bacteria complex after the first 5 subjects.

Polymerase chain reaction of the IMS-separated bead-bacteria complex was performed using primers specific to the *H pylori* 16s ribosomal RNA (rRNA) gene as previously described.[18] A 139–base pair (bp) band on agarose gel electrophoresis indicated the presence of *H pylori* in the sample. Negative controls included sterile distilled water and immunomagnetic beads without added samples. In inoculation experiments, IMS PCR detected 33 *H pylori* organ-

isms/mL in stools and 3 organisms/mL in vomitus.[14]

Detection of *H pylori* in Air Samples

To establish air sampling methods, we aerosolized *H pylori* (10^9 organisms/mL) in a biosafety hood while sampling air at varying intake speeds onto plates and filters. Culture could detect between 10^6 and 10^7 aerosolized organisms, with heaviest growth occurring at a 1.7 m^3/h intake speed. Filter strips were placed in 100 µL of sterile water; half were then sonicated. Filters then underwent 6 cycles of freeze-thaw lysis. We tested the filter solutions for the 16s rRNA gene using PCR as described herein. Fluoropore filters (Millipore, Bedford, Mass) consistently yielded *H pylori* without sonication (sensitivity = 10^3 organisms) and were chosen for this study.

Fingerprinting of Isolates and Confirmation of *H pylori* Identity

Isolates were confirmed as *H pylori* biochemically (oxidase, urease, and catalase positive) and by morphology under light microscopy. From each positive culture, we subcultured 1 colony and amplified the *H pylori* 16s rRNA gene as described herein.[18] If the 16s rRNA gene amplified, we then fingerprinted the isolate using repetitive extragenic palindromic (REP) PCR as previously described.[19,20] Repetitive extragenic palindromic PCR fingerprints of isolates from vomitus, stools, air, and saliva within and among subjects were compared.

To confirm species identity, isolates with unique REP PCR fingerprints were sent in a blinded fashion to Midi Labs (Newark, Del) for sequencing of the first 500 bp of the 16s rRNA gene.[21] For PCR-positive, culture-negative samples, the 139-bp 16s rRNA amplicon was sequenced in our laboratory.

RESULTS

The mean age of the 16 infected subjects was 38.7 years (range, 22-53 years) and 9 (56.3%) were women. The mean age of the 10 uninfected subjects was 39.0 years (range, 29-49 years) and 6 (60%) were women.

Stool Results

Stools collected prior to administration of cathartic from all 16 infected and 10 uninfected subjects were negative by culture for *H pylori*. In 5 infected subjects but no uninfected subjects, IMS PCR detected the *H pylori* 16s rRNA gene (TABLE 1).

From infected subjects, we collected 121 cathartic stools (mean per subject, 7.6; range, 4-13), of which 115 were cultured; at least 4 samples were cultured from each subject (TABLE 2). Cultures from 2 subjects were unevaluable due to fungal overgrowth; cultures from 7 (50%) of the remaining 14 subjects yielded *H pylori*. Stools passed late in catharsis were more likely than early stools to grow the organism. The amount of *H pylori* shed in stool was quantifiable in 16 of 37 culture-positive stools from 5 subjects; the number of CFU/mL ranged from 5 to 2125. In 1 subject in whom sequential stool cultures were quantified, the amount of *H pylori* appeared to increase in the later samples collected (500 CFU/mL in sample 5, 725 CFU/mL in sample 6, and 2124 CFU/mL in sample 7).

Table 1. Results From Stools, Saliva, Vomit, and Air From 16 Subjects Infected With *Helicobacter pylori*

	Precatharsis Stool		Cathartic Stool		Preemesis Saliva		Postemesis
Subject	Culture	IMS PCR	Culture	IMS PCR	Culture	IMS PCR	Culture
13	−	⊕	+	+	−	−	+
20	−	−	+	+	−	−	+
26	−	−	NE†	−	−	−	−
27	−	−	−	⊕	−	−	−
31	−	⊕	−	−	−	−	−
33	−	+	−	−	−	+	−
48	−	−	+	+	−	⊕	⊕
56	−	⊕	−	⊕	+	+	+
57	−	−	+	−	+	⊕	−
61	−	⊕	−	⊕	−	⊕	+
62	−	−	+	+	−	−	−
75	−	−	+	+	+	⊕	+
85	−	−	NE	−	−	−	−
86	−	−	−	⊕	−	+	+
106	−	−	+	+	−	−	+
113	−	−	−	⊕	−	−	+
Total positive (%) [95% CI]‡	0/16 (0) [0.0%-24.0%]	5/16 (31.3) [12.1%-58.5%]	7/14 (50.0) [24.0%-76.0%]	11/16 (68.8) [41.5%-87.9%]	3/16 (18.8) [5.0%-46.3%]	7/16 (43.8) [20.8%-69.5%]	9/16 (56.3) [30.6%-79.2%]

*Circles indicate isolates or amplicons from which a portion of 16s ribosomic RNA gene was sequenced to confirm species indentity; shaded boxes, successfully subcultured organisms that were fingerprinted using repetitive extragenic palindromic polymerase chain reaction; plus and minus signs, positive and negative results on any sample, respectively; and IMS PCR, immunomagnetic separation polymerase chain reaction.
†NE indicates not evaluable; cultures from these subjects were contaminated by fungus and were not interpretable.
‡Fleiss quadratic 95% confidence intervals (CIs).[22]

By IMS PCR, 11 of 16 subjects had at least 1 cathartic stool positive for *H pylori*. Stools excreted both early and late during catharsis were equally likely to have the *H pylori* 16s rRNA gene fragment detected. Among the 5 subjects without *H pylori* DNA detected during catharsis, 2 had had *H pylori* DNA detected in stool prior to catharsis; an additional subject with negative IMS PCR results had a positive stool culture. Thus, stools from 14 (88%) of 16 subjects showed evidence of potentially viable *H pylori*. The 2 subjects without *H pylori* detected in their stools were those with contaminated culture plates.

Emesis and Saliva Results

We collected 85 vomitus samples from infected subjects (mean per subject, 5.3; range, 3-8). Five samples were contaminated with fungus and could not be evaluated; the remaining 80 samples all grew *H pylori*. Cultures could be quantified from 38 samples representing 14 subjects. The number of CFUs per specimen was high, with greater than 1000 CFU/mL of vomitus in 31 samples and greater than 10 000 CFU/mL in 11 samples (range, 10-30 000 CFU/mL). Immunomagnetic separation PCR detected the *H pylori* 16s rRNA gene in all samples. The uninfected subject who was administered ipecac vomited 3 times; all cultures and PCR assays from this subject were negative for *H pylori*.

Saliva prior to emesis was positive for *H pylori* by culture in 3 infected subjects (18.8%) and by IMS PCR in 7 infected subjects (43.8%), including the 3 subjects with positive cultures. A half hour after termination of emesis, saliva cultures were positive for *H pylori* from 9 infected subjects (56.3%) and IMS PCR results were positive in 8 infected subjects (including 7 of the subjects with positive cultures). Quantities of *H pylori* in postemesis saliva tended to be low (4 quantified cultures had counts ranging from 50-500 CFU/mL). Saliva samples from the uninfected controls were all negative for *H pylori* both by culture and by IMS PCR. Saliva from 1 uninfected subject who underwent emesis was again negative for the organism after vomiting.

Saliva	Emesis		Sampled Air	
IMS PCR	Culture	IMS PCR	Culture	Filter PCR
−	+	+	+	−
−	+	+	⊕	+
−	⊕	+	−	−
−	⊕	+	+	−
−	⊕	+	−	−
⊕	⊕	+	−	−
+	+	+	−	−
+	⊕	+	+	+
−	+	+	+	−
+	⊕	+	−	−
−	⊕	+	−	−
+	+	+	⊕	−
−	+	⊕	−	−
+	⊕	+	−	+
+	⊕	+	−	+
+	⊕	+	−	−
8/16 (50.0) [25.5%-74.5%]	16/16 (100.0) [75.9%-100%]	16/16 (100.0) [75.9%-100%]	6/16 (37.5) [16.3%-64.1%]	4/16 (25.0) [8.3%-52.6%]

Air Sampling Results

Air sampled prior to onset of vomiting did not yield *H pylori* by culture or PCR. After onset of vomiting, air sampled 0.3 m away from 6 subjects grew *H pylori*. In 5 of these 6 instances, the positive culture coincided with the first episode of vomiting; in the sixth instance, the positive culture coincided with the fifth bout of vomiting. In 2 of the air culture–positive cases, the filter was also positive by PCR. Filters were additionally positive from 2 cases with negative air cultures. No sample obtained 1.2 m from the subject yielded *H pylori*.

Fingerprinting and Strain Identification

At least 1 *H pylori* isolate was available for fingerprinting from 14 of the 16 subjects; 2 subjects had isolates available from all 4 types of samples (stool, vomit, air, and saliva), 4 from 3 types, 4 from 2 types, and 4 from only 1 type. Fingerprints differed among subjects, including between a wife-husband pair (TR26 and TR31) but were identical within subjects (FIGURE).

All 14 unique REP PCR isolates obtained from the 14 subjects were confirmed as *H pylori* by sequencing of 500 bp of the 16s rRNA gene with no more than 0.82% difference from the reference strain. For IMS PCR–positive, culture-negative samples, the 16s rRNA amplicon sequence was consistent with *H pylori* in 14 of 20 samples (Table 1). For the remaining 6 IMS PCR–positive, culture-negative samples, we were unable to reamplify sufficient DNA to perform sequence analysis.

COMMENT

In this study, we found that *H pylori* can be cultured from both vomitus and stools of healthy *H pylori*–infected persons. *Helicobacter pylori* was often present in high quantities in vomitus, with as many as 30 000 CFU/mL of sample. Since the sensitivity of our vomitus culture was between 0.1% and 1%, we estimate that more than 10^6 organisms may be present in each milliliter vom-

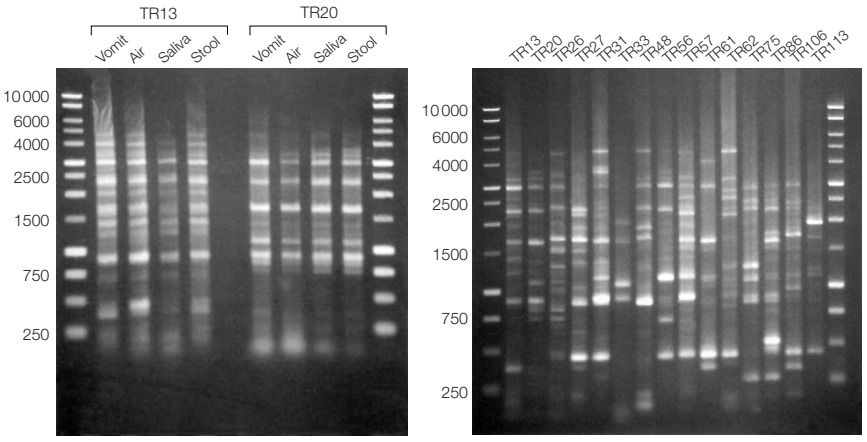
ited. Thus, emesis could be a potent mechanism for discharging millions of *H pylori* into the environment. Although patterns of disease transmission by vomiting have not been systematically studied, one would expect risk factors for transmission by vomitus to be similar to those documented for *H pylori*, eg, close living quarters, many siblings, and poor household sanitation and hygiene.[23,24] The few documented cases of acute *H pylori* infection support gastric-oral transmission. Mitchell and colleagues[25] reported acute *H pylori* infection in 1-year-old twins 3 weeks following a sustained vomiting illness in their *H pylori*–infected mother. A second acute infection was reported in a researcher who routinely processed gastric juice.[26] Possible gastric-oral transmission of *H pylori* was also reported following mouth-to-mouth resuscitation of an infected person who had vomited.[27]

Table 2. Results of Cultures and IMS PCR From Induced Stools of 16 *Helicobacter pylori*–Infected Subjects*

	Sequential Stool Sample No.												
Subject	1	2	3	4	5	6	7	8	9	10	11	12	13
13	−	−	−	−	−	−	−	−	+	+	+		
20	−	−	−	+	+	+							
26	Unevaluable†												
27	−	−	−	−	−								
31	−	−	−	−	−	−							
33	−	−	−	−	−	−							
48	−	−	−	−	−	−	−	−	−	−	+	−	−
56	−	−	−	−	−	−	−	NC	NC	−	NC	NC	−
57	−	−	+	−	+	+							
61	−	−	−	−	−	−	−	−					
62	−	+	+	+	+	+	+						
75	−	−	−	−	−	+	+						
85	Unevaluable†												
86	−	−	−	−									
106	−	−	−	+	+	+	+						
113	−	−	−	−	−	−	−						

*Plus sign indicates positive culture for *H pylori*; minus sign negative culture; shaded boxes, positive immunomagnetic separation polymerase chain reaction (IMS PCR); and NC, not cultured.
†Cultures from these 2 subjects were unevaluable due to contamination of plates by fungus. In both of these subjects, IMS PCR results were also negative from all samples.

Figure. *Helicobacter pylori* Fingerprints Within and Among Subjects



Left, Example of repetitive extragenic palindromic polymerase chain reaction fingerprints of isolates from different specimens within individual patients. In the 2 examples shown, *H pylori* isolates from vomitus, stools, air, and saliva within 2 individual subjects (TR13 and TR20) had identical fingerprints. All *H pylori* fingerprints within individual subjects—regardless of specimen of origin—were identical to one another. Right, In contrast, each subject's *H pylori* had its own unique fingerprint.

The process of vomiting also dispersed *H pylori* into the air. Although other gastrointestinal tract pathogens, notably the small, round, structured Norwalk-like viruses, can be transmitted by aerosol during episodes of vomiting, we doubt this is a common mode for *H pylori* transmission.[28,29] The short duration of contaminated aerosol (in the first minutes of the first episode of vomiting) and the limited dispersion of organisms (less than 1.2 m) makes aerosol exposure unlikely.

Helicobacter pylori DNA has frequently been amplified from both saliva and dental plaque,[10,11,30] but only rarely has *H pylori* been cultured from the mouth.[9,30,31] We recovered *H pylori* from saliva before emesis in 19% of subjects and after emesis in 50% of subjects. To date, there is little epidemiological data to support oral-oral transmission. Dental workers have similar prevalence of *H pylori* as the average population.[32] Most married couples demonstrate little concordance of infection or strain type[33,34] and treated patients are not reinfected by their untreated infected spouses.[35] Thus, it remains to be seen whether organisms in the mouth, which were typically present in low quantities compared with in vomitus, represent a significant source of transmission.

Helicobacter pylori was less reliably cultured from stools than from vomitus. This may, in part, be due to the lower sensitivity of stool culture. In 50% of subjects, however, *H pylori* could be cultured from feces in the setting of rapid gastrointestinal tract transit.

It can be argued that cathartic-induced diarrhea and emetic-induced vomiting do not mimic gastroenteritis. It is likely that *H pylori* needs to be rapidly excreted from the proximal gastrointestinal tract to be found viable in

stools. Indeed, the lack of regulatory genes in *H pylori* implies that the organism cannot survive for long periods outside its normal environment.[36] Yet, only pathogens involving the small bowel induce the watery diarrhea seen with sodium phosphate administration. This being the case, colitic forms of gastroenteritis and gastroenteritis with relatively slower intestinal transit may not transmit *H pylori*. Vomitus, on the other hand, was so uniformly contaminated with high amounts of *H pylori* that it is difficult to envision circumstances in which it would not be infectious. This also raises the question of whether persons with chronic gastric regurgitation or frequent vomiting from other medical conditions are high-risk *H pylori* transmitters.

In this study, we evaluated only healthy asymptomatic adults and found that viable *H pylori* was excreted into the environment by all infected subjects. Given the large number of infected hosts worldwide, it is remarkable that so many remain uninfected. Barriers to acquiring infection—both intrinsic to the host (eg, high gastric acidity, good nutrition) and extrinsic to the host (eg, household and public sanitation and personal hygiene)—may account for this phenomenon. We postulate that a declining incidence of gastroenteritis that occurs as countries make the transition from developing to developed may also contribute to the observed decline in *H pylori* infection in industrialized nations.[37,38] Epidemiological investigations within households of persons with gastrointestinal tract illness may provide important clues to understanding and controlling *H pylori* transmission.

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