A New Model for the Transmission of *Helicobacter pylori*: Role of Environmental Reservoirs as Gene Pools to Increase Strain Diversity

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Twenty-five years after the first successful cultivation and isolation of *Helicobacter pylori*, the scientific community is still struggling to understand the way(s) this bacterium is transmitted among the human population. Here, both epidemiologic and microbiologic evidence addressing this matter is reviewed and explored to conclude that most *H. pylori* successful colonizations are derived from direct person-to-person contact and that even though exposure of humans to *H. pylori* from environmental sources is a very common event, in most occasions the host is able to fight off infection. In addition, under a new model developed here, we propose that the near elimination of environmental reservoirs is the main responsible for the lower prevalence observed in the more industrialized countries by acting on two levels: by decreasing the number of direct infections and by diminishing the number of intraspecies recombination events for producing strain variation within *H. pylori*.

Keywords *Helicobacter pylori*; Epidemiology; Microbiology; Water

Helicobacter pylori is a Gram-negative ε-proteobacterium closely related to *Campylobacter* spp. It has now been well established that infection of the human host with this microorganism is a risk factor for the development of gastric-related diseases (reviewed in Malfertheiner et al. 2006). In fact, much of the research on *H. pylori* has been concerned with the determination of its role in human condition and on the identification and characterization of virulence factors that contribute to disease development (e.g., Blaser and Atherton 2004; Prinz, Hafsi, and Voland 2003; Figueiredo, Machado, and Yamaoka 2005).

Other issues that have been receiving a great deal of attention are the prevalence and epidemiology of infection (e.g., Kikuchi and Dore 2005; Queiroz and Luzza 2006; Brown 2000). The first one is essential to understand the extent of the problem for human populations, whereas the second tries to relate presence of infection with a number of factors that are relevant for acquisition. Even though these types of studies provide a good indication on how a microorganism is transmitted, they usually require subsequent confirmation. In general, verification can be accomplished by using microbiological techniques to grow and isolate the microorganism from suspected reservoirs. However, the latter has failed to provide definitive conclusions on the suspected routes

and vehicles of transmission, a situation that can be explained by some unique features of *H. pylori* interaction with human individuals and the bacterium's physiology. For instance, infection of the bacterium usually goes by unnoticed for the entire life of the host, and even when it causes health concerns, first symptoms generally take a long time to occur after infection (Brown 2000). This implies that small episodes of transmission and even possible outbreaks of the bacterium, if any, would be unnoticed by public health authorities. Another hindering aspect is that *H. pylori* is relatively complicated to isolate from any natural system (except for the human gastrointestinal tract) under currently used culture media (Andersen and Wadstrom 2001), especially if the environment houses other bacteria. Whether the bacterium enters or not a viable but non-culturable (VBNC) state is a matter of discussion (Chaput et al. 2006; Bumann et al. 2004), and even more debatable is if under stressful conditions the bacterium can infect the human host. Addressing all these issues is of extreme importance, as they may lead to the development of prophylactic measures to decrease the prevalence observed across the world.

This review will start by indicating prevalence levels of *H. pylori* in the human population. Then, possible routes of transmission will be described, together with the presentation of both epidemiological and microbiological data supporting or dismissing each individual route. Finally, a discussion based on the evidence provided will conclude about the most likely routes of transmission, develop a model to explain the observed decrease in prevalence in industrialized countries and suggest what needs to be performed in order to advance our understanding.

PREVALENCE OF *H. pylori* ACROSS THE WORLD

H. pylori infection occurs worldwide, but significant differences in prevalence have been found both within and between countries (Mitchell 2001). Generally, the overall prevalence is higher in countries of underdeveloped regions, such as Africa and Asia, than in the more developed countries in Western Europe and North America (Figure 1). Overall, *H. pylori* prevalence is decreasing as a result of improved sanitary conditions and treatment procedures (Rehnberg-Laiho et al. 2001).

It is now well known that *H. pylori* infection is mostly acquired in childhood, and that by the age of 10 more than 50% of children worldwide carry the organism (Pounder and Ng 1995). In addition, adults might also acquire infection but at much lower rates (Kivi and Tindberg 2006). A number of studies have suggested that transient infections might occur and even be quite common (e.g., Goodman et al. 2005; Haggerty et al. 2005), but the significance of these results are still under dispute due to the specificity and sensitivity of the tests used (Perry and Parsonnet 2005; Nugalieva, Opekun, and Graham 2006). Recent evidence has also showed that multiple *H. pylori* strains and other Helicobacter spp. are able to infect simultaneously the GI tract of one individual (Van den Bulck et al. 2005; Fritz et al. 2006; De Gusmao et al. 2000; Daugule et al. 2003; Ghose et al. 2005; Kang and Blaser 2006; Israel et al. 2001). Even in cases where multiple strains may occur as a result of intensive genetic recombination of a single ancestral strain, it is recognized that the ancestor had to be in contact with other strains (Delport et al. 2006).

FIG. 1. Prevalence of *H. pylori* in human populations worldwide. The map was based on data collected by Lunet et al. (Lunet and Barros 2003) and completed with other studies (Torres et al. 2003; Nurgalieva et al. 2002; Bakka and Salih 2002; Erreimi et al. 1999; Boyanova et al. 1994; Koch et al. 2005; Con et al. 2006; Carrillo et al. 2005; Carrillo 2005; Hoodeh et al. 2005; Aguemon et al. 2005; Hoang et al. 2005; Vollaard et al. 2006). The criteria used for the selection of new studies is also described in (Lunet and Barros 2003). N. D. stands for places where a consistent study about *H. pylori* prevalence has not yet been conducted.

In accordance with these data, the classical scenario where the host is generally exposed only once to the bacterium, but where colonization is usually successful appears to be losing ground to a scenario where there is constant exposure to *H. pylori* but where the balance between the host and the bacterium tends to favor the elimination of the microorganism or the colonization by multiple strains. This latter scenario implies that the decreasing levels of *H. pylori* in the human GI tract observed in developed countries are not due to a decreasing number of exposure events between the microorganism and the host but instead to increased resistance of the host to the bacterium or "less fit" *H. pylori* in contact with the host.

ROUTES OF INFECTION

Numerous epidemiological studies have been conducted to identify the factors influencing transmission of this pathogen. Socioeconomic status is clearly the most important determinant for the development of *H. pylori* infection, with poorer/lower social classes exhibiting much higher prevalence (Mitchell 2001), which is also in accordance with differences found between underdeveloped and developed countries described in the previous section. This factor encompasses conditions such as levels of hygiene, density of living, sanitation, and educational opportunities, which have all been individually identified as markers of the bacterium presence.

Largely based on epidemiological and microbiological evidence, several routes of transmission have been conjectured (Figure 2). Person-to-person transmission is widely seen as the most probable route of infection, mainly because of the apparent failure to consistently isolate *H. pylori* in places other than the human GI tract and of the perception that lower transit time between different hosts would certainly be favorable for the bacterium. Furthermore, numerous epidemiological studies have consistently identified domestic overcrowding and infection of family members as a risk factor for *H. pylori* transmission. Roma-Giannikou and colleagues (2003) found a strong homology of the *H. pylori* genome in infected members of the same family, and clustering of *H. pylori* infection in families has been widely reported in other studies (e.g., Ma et al. 1998). Although these studies support the hypothesis of person-to-person transmission, exposure of a family to an alternative common source still remains a possibility.

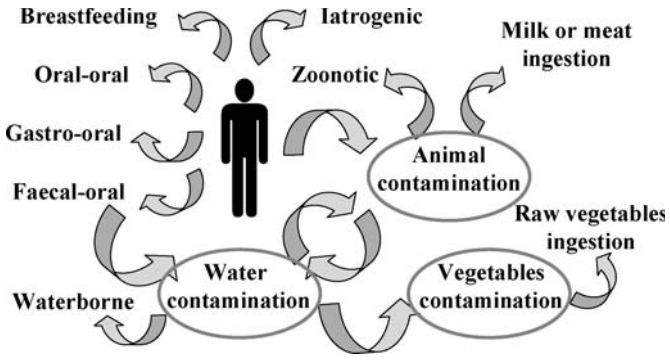

FIG. 2. Suggested transmission routes for *H. pylori*. Five of the proposed pathways are representative of direct person-to-person transmission (breastfeeding, iatrogenic, oral-oral, gastro-oral, and fecal-oral), whereas the remaining four require an environmental reservoir in between. Possible reservoirs outside the human host are marked with a red circle.

The most relevant pathways of person-to-person transmission encompass the gastro-oral, oral-oral, and fecal-oral routes. Breastfeeding and iatrogenic transmission are also included as alternative ways for the dissemination of the pathogen. In addition, there are at least three possible vectors that have been suggested to sustain the bacterium in viable form: water, food, and animals. Most authors agree that the relative importance of these routes in the transmission of the bacterium is likely to vary between developing and developed countries (Perez-Perez, Rothenbacher, and Brenner 2004; Megraud 2003). The most relevant in overall terms are now addressed in detail.

Gastro-Oral Transmission

It has been suggested that exposure to microscopic droplets of gastric juice during endoscope manipulation could explain an higher prevalence of infection in gastrointestinal endoscopists (Hildebrand et al. 2000), but the gastro-oral transmission has been postulated mainly for young children, among whom vomiting and gastro-esophageal reflux are common. In a recent epidemiological study, exposure to an infected household member with gastroenteritis and vomiting episodes was associated with a 6.3 fold increased risk of new infection (Perry et al. 2006). It is important to realize, however, that because vomiting episodes might cause an increased risk of the presence of *H. pylori* in the oral cavity, this type of study does not discriminate whether the transmission is gastro-oral or oral-oral.

In a study by Parsonnet et al. (Parsonnet, Shmuely, and Haggerty 1999), vomitus from infected subjects and surrounding air were sampled for *H. pylori*. All vomitus samples were positive (often recovering the bacterium in high quantities), and even the surrounded air tested positive for 37.5% of the cases. Successful cultivation of *H. pylori* from vomitus was also obtained in two other studies (Leung et al. 1999; Young et al. 2000). Amazingly, there is a blatant lack of data on the survival/culturability time of the bacterium in gastric juice, and as such, it is not possible to estimate for how long the infectious state might last on these conditions and to establish comparisons with culturability times obtained for other conditions.

On the other hand, the discovery of enterohepatic *Helicobacter* species might challenge the importance of a gastro-oral (and an oral-oral) route (Solnick and Schauer 2001). As the name suggests, these bacteria have been identified in the intestinal tract and/or the liver of humans, other mammals, and birds, which implies a more unlikely presence for them in the oral cavity and stomach. How these bacteria are transmitted is something that has been little studied, but most works appear to support a fecal-oral route (Livingston et al. 1998; Bohr et al. 2006; Fox et al. 1992). The question to be asked here is whether the phylogenetic

proximity to *H. pylori* would imply that transmission routes are similar.

Oral-Oral Transmission

The oral cavity has been considered to be a suitable reservoir for *H. pylori* subsistence, and oral-oral transmission has therefore been suggested to occur with kissing or other contact with infected saliva, the use of chopsticks by Chinese immigrants or, as it happens in some ethnic backgrounds, from mothers to their babies as they pre-masticate their food. The role of the oral cavity has been extensively reviewed by others (Megraud 1995; Dowsett and Kowolik 2003; Luman 2002), and as such, we will only update information and state the most relevant conclusions. Identical strains of the pathogen have been detected by polymerase chain reaction (PCR) in the mouth and stomach of symptomatic infected individuals (Khandaker et al. 1993), and in these populations detection of *H. pylori* in the oral cavity by PCR is in fact very common (Dowsett and Kowolik 2003). Nevertheless, studies conducted afterwards using similar techniques indicated that the oral cavity does not favor prolonged colonization of *H. pylori* in populations with high prevalence of infection when the individuals are asymptomatic, and concluded that colonization of the mouth is only transient and occurs after vomiting (Dowsett and Kowolik 2003; Olivier et al. 2006). Similarly, isolation and cultivation of the microorganism has been sporadic and related to transitory regurgitations of the microorganism from the stomach into the mouth (Dowsett and Kowolik 2003; Luman 2002). Microbiological studies on the culturability of *H. pylori* on a buffer containing a peroxidase system with high concentrations of H_2O_2 (to simulate saliva), showed that after 1 hour at 37°C the bacterium started to be inhibited, but this inhibition was not noticed when the buffer system was added to real human saliva (Haukioja et al. 2004). Luman et al. compared the genotypes of *H. pylori* isolated from patients and their spouses by PCR-restriction fragment length polymorphism and found very little similarity (Luman et al. 2002). It is however possible that several mechanisms, such as point mutations and intragenic recombination, could enhance strain diversity once the infection is acquired.

Fecal-Oral Transmission

It has been suggested that the fecal-oral route for *H. pylori* transmission is very unlikely due to the contact with human bile, to which it is very sensitive, during the passage through the intestine (Hanninen 1991; Mitchell et al. 1992). One epidemiological study appears to support the view that this transmission mode is less common than gastro-oral or oral-oral, by showing that exposure to an infected household member with diarrhea elevated, but not significantly, the risk for new infection (Perry et al. 2006).

However, the fact that *H. pylori* is able to colonize the duodenum (upper part of the small intestine) in areas of gastric metaplasia, appears to be an inconsistency, and has raised some questions about the exact effect of the passage of the microorganism through the intestine (Han et al. 1996). Well-established detection methods based on PCR or enzyme-linked immunoassays systematically identify the presence of the microorganism (e.g., Ndip et al. 2004; Kabir 2001; Notarnicola et al. 1996; Queralt, Bartolome, and Araujo 2005), but growth of the bacterium using culture methods has been more elusive, and achieved most of the times in individuals with accelerated gut transit time (Luman 2002; Dowsett and Kowolik 2003).

Breastfeeding

The detection by PCR of *H. pylori* in breast milk has also raised the possibility of breastfeeding as a route of transmission (Kitagawa et al. 2001), even though earlier studies stated that infants born from *H. pylori*-positive women are not more likely to acquire the infection (Blecker et al. 1994). The contamination of milk could be possible if the bacterium survived in nipples or fingers. However, most epidemiological studies appear not to find any correlation between breastfeeding and *H. pylori* acquisition (Table 1). In fact, a few of them actually mention breastfeeding as a protection practice against the microorganism (Pearce et al. 2005; Ertem, Harmanci, and Pehlivanoglu 2003; Malaty et al. 2001). Survival studies indicate that the bacterium remains culturable in commercial pasteurized milk for 5 days at 4°C and an inoculum concentration of $\approx 10^4$ CFU/mL (Poms and Tatini 2001). It is likely, however, that this relatively long time of survival is related to the low temperatures at which the experiment was carried out.

Iatrogenic Transmission

Acquisition of *H. pylori* by patients submitted to upper endoscopy, i.e., iatrogenic transmission, is supported by three out of four epidemiological studies (Table 1). This value of 75% should nevertheless be interpreted with care as in all but one study, the population that was followed was medical staff from gastrointestinal endoscopy units and as shown above, this group might acquire infection by, for instance, the gastro-oral route and not by direct contact with endoscopes. *H. pylori* has been consistently detected by culture in endoscopes after their use in infected patients (Nurnberg et al. 2003; Brown et al. 2005; Katoh et al. 1993), but adequate disinfection procedures are thought to greatly reduce (or even eliminate) the transmission risk for this microorganism (Cronmiller et al. 1999). Back in 1995, Tytgat estimated a transmission frequency of approximately 4 patients per 1000 endoscopies when the infection rate in the endoscoped population was about 60% (Tytgat 1995).

Zoonotic Transmission

Including contact with animals as a possible transmission mode is an obvious reasoning, as zoonotic transmission represents one of the leading causes of illness and death from infectious disease worldwide. As indicated in Table 1, most epidemiological studies appear to support the role of animals in the

TABLE 1

Number and percentage of epidemiological studies that considered certain routes of transmission as risk factors, together with survival data of the bacterium for each of the pathways

Type of transmission	Positive association[a]	Ref(s)	*H. pylori* survival data[c]	Ref(s)
Gastro-oral	>100 (100%)[e]	(e.g., Rodrigues et al. 2004; Lin et al. 1999; Rothenbacher et al. 1999)	Spiking experiments not available.	—
Oral-oral			It remains culturable for at least 1 hour at 37 °C.	(Haukioja et al. 2004)
Fecal-oral			Spiking experiments not available.	—
Iatrogenic	3 (75%)[b]	(Goh, Parasakthi, and Ong 1996; Wu et al. 1996; Mastromarino et al. 2005; Noone, Waclawski, and Watt 2006)	Endoscopes challenged with 10^8 CFU/mL were still contaminated after manual cleaning but not after disinfection.	(Cronmiller et al. 1999)
Breastfeeding	1 (17%)	(Malaty et al. 2001; Rothenbacher, Bode, and Brenner 2002; Ertem, Harmanci, and Pehlivanoglu 2003; Pearce et al. 2005; Nguyen et al. 2006; Rodrigues et al. 2006)	Culturable in pasteurized milk for 5 days at 4°C.	(Poms and Tatini 2001)
Zoonotic	7 (64%)[d]	(Goodman et al. 1996; Lindo et al. 1999; Jimenez-Guerra, Shetty, and Kurpad 2000; Herbarth et al. 2001; Plonka et al. 2006; Bode et al. 1998; Brown et al. 2001; Garcia et al. 2006; Cataldo et al. 2004; Dore, Bilotta et al. 1999; Begue et al. 1998)	Largely dependable on the animal and location studied (see main text).	(Imamura et al. 2003; Grubel et al. 1997; Osato et al. 1998)
Water	13 (76%)	(Garcia et al. 2006; Goodman et al. 1996; Ilboudo et al. 1998; Yamashita et al. 2001; Lin et al. 1999; Lindo et al. 1999; Jimenez-Guerra, Shetty, and Kurpad 2000; Olmos et al. 2000; Herbarth et al. 2001; Lyra et al. 2003; Klein et al. 1991; Begue et al. 1998; Aguemon et al. 2005; Al-Shamahy 2005; Iso, Matsuhisa, and Shimizu 2005; Ahmed et al. 2006; Mitipat et al. 2005)	Survival depends on temperature. At 4°C it remains culturable for 20–25 days; at 15°C for 10–15 days and at 24°C for 6–10 hours.	(Shahamat et al. 1993; Azevedo et al. 2004; Adams, Bates, and Oliver 2003)
Food	6 (75%)[d]	(Goodman et al. 1996; Ilboudo et al. 1998; Russo et al. 1999; Constanza et al. 2004; Turkdogan et al. 2005; Hopkins et al. 1993; Begue et al. 1998; Ahmed et al. 2006)	Largely dependable on the type of food (see main text)	(Gomes and De Martinis 2004; Poms and Tatini 2001)

[a] By positive association we consider studies where the impact of factors related to the transmission pathway were statistically significant.

[b] Because epidemiological data on effective transmission of *H. pylori* to patients is scarce, studies addressing the contamination of medical staff from gastrointestinal endoscopy units were also included.

[c] As assessed by cultivability methods, which might in fact underestimate the true survival time of the bacterium.

[d] Discrimination between animals and types of food can be found in the main text.

[e] Epidemiological studies consistently point to domestic overcrowding and infection of family members (or similar) as a risk factor for *H. pylori* acquisition.

acquisition of *H. pylori*, but the extent of this support depends on the animals under study. Considered vectors include cows (Fujimura et al. 2002), sheep (Dore et al. 2001), cockroaches (Imamura et al. 2003), houseflies (Osato et al. 1998), and domestic pets (Boomkens et al. 2004).

In the first two cases, the suspected route of transmission is mainly by the ingestion of contaminated raw milk. Milk could become contaminated when the breast of a cow or sheep is in contact with feces in the soil. Epidemiologic data has shown higher prevalence in shepherds and their families than in the general population (Dore et al. 1999; Plonka et al. 2006). The detection of the bacterium in animal milk is described ahead (see food ingestion section).

Inamura et al. (Imamura et al. 2003) suggested that cockroaches, which usually live in unsanitary environments, may contaminate foods and food containment areas such as pantries. The authors studied the survival of *H. pylori* on the external surfaces (legs and body) and excreta of *H. pylori*-exposed cockroaches and found that the microorganism was culturable from the excreta of the exposed group for 24 h postchallenge, but not from the external surfaces. A similar study was also performed with houseflies (Grubel et al. 1997). In this case, *H. pylori* was recovered from external surfaces for up to 12 h and from gut and excreta for as long as 30 h postchallenge. The negative detection after 30 h was attributed to the appearance of other Gram-negative bacteria that overgrew the cultures. However, when this study was repeated exposing the houseflies to *H. pylori*-contaminated human feces instead of *H. pylori* grown on agar plates, the microorganism was not cultured from any of the locations (Osato et al. 1998).

Epidemiological studies showed controversial results in respect to the risk of the presence of domestic animals in the household (Table 1) (e.g., Bode et al. 1998; Lindo et al. 1999; Kearney and Crump 2002). *H. pylori* has not been found in dogs and only very rarely in cats' stomachs (ElZaatari et al. 1997; Neiger and Simpson 2000), and it has been suggested that the presence in animals is of human origin (Cittelly et al. 2002; ElZaatari et al. 1997). Recent work has identified *H. pylori* by PCR in the bile of cats, thus increasing the chance of this animal as a vector (Boomkens et al. 2004). It is now known that nearly each animal is colonized by its own endogenous *Helicobacter* spp. Like *H. pylori*, that has co-evolved with humans to be highly specialized in the colonization of the human GI tract (Falush et al. 2003), these bacteria have specialized in colonizing the GI tract of their specific natural host. In the model where only one strain colonizes the stomach of a mammal, *H. pylori* would find fierce competition by these other *Helicobacter* spp. in search for essential nutrients and not subsist. With the emergence of a multiple infecting strains and species model for the same host it is more credible that *H. pylori* is also a zoonotic agent.

Water Ingestion

A large number of epidemiological studies have investigated drinking water, or drinking water-related conditions, as a risk factor for *H. pylori* infection (Table 1). Although a few studies report the absence of an association between prevalence of *H. pylori* and water quality, the majority of the other studies support a relationship between these parameters.

Concomitantly, molecular methods such as FISH, PCR and antibody assays, were able to detect the presence of the bacterium in water and water-associated biofilms from wells, rivers and water distribution networks (e.g., Bragança et al. 2005; Piqueres et al. 2006; Fujimura, Kato, and Kawamura 2004). However, when suspended in water, *H. pylori* has a very low culturability time when compared to other waterborne pathogens. In fact, several studies report culturability times of less than 10 hours for *H. pylori* (Adams, Bates, and Oliver 2003; Azevedo et al. 2004; Shahamat et al. 1993) at temperatures over 20°C which compares to culturability times of more than 40 days for *Escherichia coli* and *Salmonella typhimurium* at the same temperature. While *H. pylori* culturability usually ends after little time in water and water-exposed biofilms (Azevedo, Pacheco et al. 2006; Azevedo, Pinto et al. 2006), Shahamat et al. (Shahamat et al. 1993) determined that total cell counts did not decrease for much longer periods (2 years at 4°C). This raises questions on the exact physiological state of the bacterium in water and in the evaluation of methods that could be more appropriate for the detection of the infectious microorganism. For instance, a nutrient shock effect was also observed when recovering the water-stressed bacterium to high nutrient medium, showing some level of *H. pylori* adaptation to this environment (Azevedo et al. 2004). Furthermore, it has been attempted to demonstrate that coccoid (cell shape associated with non-culturability) *H. pylori* induced by water is capable of colonizing the gastric mucosa and cause gastrititis in mice (She et al. 2003; Cellini et al. 1994).

Food Ingestion

At least two epidemiological studies have found a positive relationship between the consumption of uncooked vegetables and *H. pylori* transmission (Hopkins et al. 1993; Goodman et al. 1996). Raw vegetables are suspected to be vulnerable to *H. pylori* colonization when contaminated water is used for washing or irrigation. It is important to bear in mind that this route assumes that *H. pylori* is also able to survive in water and has therefore all the problems associated with this possible transmission route. No reports have been found about cultivation methods or molecular biology procedures trying to detect the microorganism from these products. Survival studies indicate that inoculated *H. pylori* (temperature: 8°C; inoculation density $\approx 10^6$–10^7 CFU/g) dropped below detection limits at 4 days in sanitized lettuce and carrot samples, and at 5 days in sterilized carrot (Gomes and De Martinis 2004). In a different study, survival of the microorganism lasted for up to 2 days in leaf lettuce (4°C; $\approx 10^2$ CFU/g) (Poms and Tatini 2001).

Milk is another type of food implicated as a possible transmission vehicle by epidemiological studies. Constanza et al. correlated infection with the intake of milk products in Mexico (Constanza et al. 2004). Conversely, an epidemiological study in

Italy reported an inverse correlation between the elevated consumption of milk and *H. pylori* prevalence (Russo et al. 1999). The differences obtained in both studies might reflect variable milk microbiological quality between these two countries. Interestingly, a recent study in Poland showed that prevalence in shepherds and their families was 20–30% higher than in farmers with no contact to sheep (Papiez et al. 2003). They understandably attributed this difference to the contact with animals (zoonosis), but failed to consider a probably higher quantity ingestion of raw milk by the shepherds and their families as a variable. Previously, in 1999, Dore et al. found similar results in a community of Sardinian shepherds and their families (Dore, Bilotta et al. 1999), but the research group went on to try and detect *H. pylori* presence in sheep milk. They were able to report the recovery of viable *H. pylori* from raw milk samples on two separate occasions (Dore, Sepulveda et al. 1999; Dore et al. 2001), but failed to confirm the survival of the microorganism after pasteurization of the milk. Furthermore, a larger screening of 400 raw sheep milk samples performed in Turkey detected no viable *H. pylori* (Turutoglu and Mudul 2002). The pathogen has also been cultured from one sample of raw cow's milk in Japan (Fujimura et al. 2002), and in the same work PCR demonstrated the presence of the *ureA* gene of *H. pylori* in 13 of 18 (72.2%) raw milk samples and in 11 of 20 (55%) commercial pasteurized milk samples.

Poms and Tatini studied the survival of *H. pylori* in other commercially available food products, such as yoghurt, chicken meat and tofu (Poms and Tatini 2001). The bacterium was cultured for 1, 2, and 7 days, respectively (4°C; $\approx 10^2$ CFU/g). Differences in the culturability could be explained by the work of Jiang and Doyle (Jiang and Doyle 1998), who, based on the effect of environmental and substrate factors on survival and growth of *H. pylori*, have stated that the microorganism usually exhibits extended survival in low acid/high moisture environments. Also, autochthonous microbiota present in the yoghurt, such as *Lactobacillus* and *Bifidobacterium*, have been shown to inhibit the survival of *H. pylori* (Wang et al. 2004; Wendakoon and Ozimek 2002).

LIKELIHOOD TO CAUSE INFECTION FOR EACH TRANSMISSION PATHWAY

The most notorious elements to be taken from the data presented in Table 1 are perhaps the amount of epidemiological evidence that appears to support all routes of transmission but breastfeeding. Even with the obvious pitfalls with this type of analysis, which include the possible existence of confounding factors not accounted for in some of the studies and the natural tendency to give relevance and present data that are significant rather than data that are not, the case for a multi-route way of transmission is compelling.

On the other hand, there is a lack of standardized microbiological plating studies for nearly all pathways. Increasing temperatures are an important factor for reducing *H. pylori* culturability in water systems, and it is likely that this effect will be observed for other microenvironments. This temperature effect partly explains why *H. pylori* has not been regularly found in the oral cavity or human feces. The relationship between culturability and effective survival of the microorganism is also yet to be fully determined, but the small number of studies available so far indicate that the latter appears to prevail for much longer than the first (She et al. 2003; Cellini et al. 1994). Once again, this information supports a role for environmental reservoirs.

But other arguments exist. Unlike for *Helicobacter* spp., infection with certain *Campylobacter* species usually induces rapid (2–5 days depending on several factors) alterations in the health of a host, causing diarrhea, abdominal pain, and fever (Moore et al. 2005; Butzler 2004). Furthermore, plating techniques have been evolving for a longer time, which implies that more effective culture media and selective supplements have already been developed (Butzler 2004). Consequently, when large outbreaks occur, researchers have systematically been able to trace back the infections to the source of contamination. With the phylogenetical proximity between both species, it should be expected that, at least in part, the behavior of both bacteria in respect to transmission could be similar, but unless *H. pylori* is indeed capable of at least limited survival in the environment, this would not hold true. In fact, *Campylobacter* spp. have long been known for being able to survive in the environment, and a recent tentative estimative on how much each route contribute to infection has estimated that 44.3% of the infections in humans were derived from livestock and the remaining from other reservoirs (Champion et al. 2005).

It is widely assumed that *H. pylori* uses genetic variation to adapt to individual hosts and specific niches within a host (Kraft et al. 2006; Kang and Blaser 2006; Suerbaum et al. 1998), and that a large proportion of temporal genomic changes within *H. pylori* are the result of intergenomic recombination (Kraft et al. 2006). It can therefore be concluded that continuous exposure to new strains is necessary for generating genomic changes in *H. pylori*. As it has been shown that the bacterium is able to retain cell integrity for at least two years in water at low temperatures, it seems very possible that the environment could serve as the main pool of genetic diversity for *H. pylori*.

In a multi-route transmission scenario, the higher contributor to prevalence is likely to be the gastro-oral transmission, as vomitus and surrounding air are the only samples from where the bacterium is able to be consistently cultured and isolated. The route classically favors spread in small units of interacting people such as families, but modern facilities such as nurseries would presumably affect prevalence (Blaser 1999). If transmission was mainly airborne, the improved cleaning procedures in these institutions would have little effect in controlling infection, leading to an increase in prevalence at some time point during the last century. As this has not been the case, we are left with direct contact with vomitus (which would be affected by improved cleaning procedures) to account for the observed decrease. Epidemiological evidence supports the view that gastro-oral is the

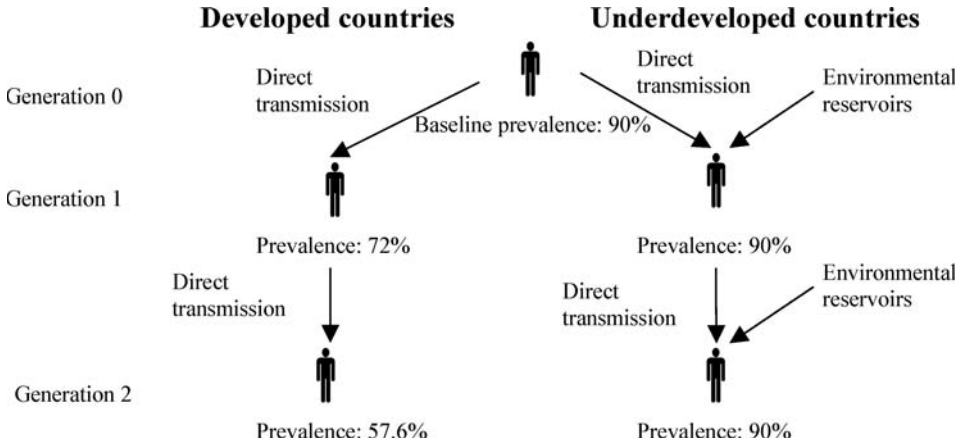

FIG. 3. Assuming that 80% of all *H. pylori* transmissions are caused by direct person-to-person contact and that the remaining 20% are caused by environmental reservoirs (either by direct infection or by maintaining strain diversity), it would only take two generations to drop from the baseline prevalence of 90% to a prevalence of 57.6%. For generations 3, 4, and 5, the values decrease to 46.1%, 36.9%, and 29.5%, respectively. It is important to notice however that only in particular situations would the environmental contribution decrease directly from 20% to 0%.

main route of transmission (Perry et al. 2006), but confirmatory studies on this issue are needed.

As gastro-oral transmission is not accountable for all cases of *H. pylori* infection, then the issue of why researchers have failed to consistently culture the bacterium outside the human GI tract has to be addressed. This situation may be attributable to either one of the following: fastidious nature of *H. pylori*; complexity of the heterotrophic microflora together with the lack of a suitable selective supplement; entry of the bacterium in the VBNC state; and the timing when sampling is performed. In either case, there are little factual differences between the fecal-oral and oral-oral route. Both are likely contributors to *H. pylori* infection but at a lower scale. On the other hand, both iatrogenic and breastfeeding appear to have at most, only residual contributions to increased prevalence.

As for environmental reservoirs, all evidence described above point that it is likely that they play a role. However, the estimates of 10^4 *H. pylori* cells for primary infection to occur is probably a very large bacterial load for *H. pylori* coming from water or food (Solnick et al. 2001), especially if we bear in mind that the bacterium has little tendency to aggregate under most environmental conditions (Azevedo, Pacheco et al. 2006; Azevedo, Pinto et al. 2006). In addition, as *H. pylori* remains culturable for longer at lower temperatures, it should be expected that colder areas would present higher infection rates and that transmission would mostly occur during winter, which is not the case. Therefore, direct infection by environmental reservoirs could be mostly attributed to zoonotic transmission. Nevertheless, water and food might have a much more subtle effect on prevalence. In this new scenario, the human body is able to recognize and fight off most attempts to colonize the human GI tract from environmental *H. pylori*, by a conjugation of three factors: low numbers of the bacterium; low physiological activity; and possibly by host resistance to most strains. Environmental reservoirs as contributors to prevalence would act however by providing a pool of genetic material, during transient passages through the GI tract, that promote the diversity of the bacterium and hence the ability to colonize all different niches in the stomach and also different individuals. With the increasing control of water parameters and food quality observed during the last century, this scenario would be able to explain the decline of *H. pylori* colonization in the developed countries (Figure 3).

CONCLUSIONS

Experience teaches that when there is a lot of conflicting evidence in one area, either the answer lies beyond the explanations advanced so far, or there is more than one correct explanation. As such, and assuming that the real answer has not escaped our attention, we suggest a hybrid solution where several routes contribute to the prevalence levels of *H. pylori* in the human population. In this scenario direct person-to-person infection (mainly including oral-oral, gastro-oral, and fecal-oral transmission) are the main contributors, together with possibly zoonotic transmission, in terms of successful colonization of the human host. The importance of food and water is mainly in terms of conserving enough genetic diversity to allow for *H. pylori* to co-evolve with humans and maintain a persistent equilibrium, a concept that is supported by mathematical modelling (Blaser and Kirschner 1999).

During the writing of this review, other valid solutions that can explain the apparent discrepancy between levels of infection in developed vs. underdeveloped countries have been evident. For instance, the classical theory where improved levels of hygiene and lower density of living in the household might still be the real reason why prevalence is receding, as is an increased resistance of human hosts to *H. pylori* due to improved diets or antibiotics intake. The model presented here should therefore not rule out the possibility of these being true, nor should it be regarded as the final solution. Nevertheless, providing it with an underlying

mathematical basis would be useful both as a confirmation and as a way of predicting future trends in prevalence.

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