Vector Potential of Houseflies (*Musca domestica*) for *Helicobacter pylori*

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The mode of transmission of *Helicobacter pylori* is unknown. Since viable bacteria have been shown to be excreted in feces from infected individuals and houseflies habitually develop and feed on excrement, we hypothesized that flies ingest and harbor *H. pylori* and, in turn, contaminate the human environment. This study examined the possible vector potential of houseflies (*Musca domestica*) for *H. pylori*. Caged houseflies were exposed to freshly grown *H. pylori* on agar plates. After a 6-h feeding period, the plates were removed and were replaced with sterile petri dishes containing a droplet of sterile brucella broth. At regular intervals, small numbers of houseflies were removed for microbiological and histological analysis, and the petri dishes were replaced with fresh sterile plates with fresh drops of brucella broth. The flies' bodies, the flies' dissected alimentary tracts, and excreta on the petri dishes were cultured for *H. pylori*, whose identity was confirmed by the urease, catalase, and oxidase reactions and Gram staining. In contrast to control flies, viable *H. pylori* could be isolated from external surfaces for up to 12 h and from gut and excreta for as long as 30 h after the initial feeding period. After 30 h other gram-negative bacteria overgrew the cultures of samples from all locations tested, rendering the selective culture of *H. pylori* colonies impossible. Histological analysis revealed *Helicobacter*-like organisms in the gut lumen and attached to intestinal epithelial cells. We conclude that houseflies can harbor viable *H. pylori* on their bodies and in their intestinal tracts. They are also able to disseminate viable *H. pylori* in excreta, and they may therefore present a significant reservoir and be a vector in the transmission of *H. pylori*.

Helicobacter pylori is a major pathogenic factor in gastroduodenal disease, including chronic type B gastritis, duodenal ulcers, and gastric adenocarcinoma (1). *H. pylori* infection is one of the commonest chronic bacterial infections of humans and affects most populations throughout the world. However, the route by which individuals become infected remains speculative. The available studies suggest the fecal-oral or oral-oral route or a common environmental source as a possible mode(s) of transmission (2, 12). Viable bacteria have been isolated from feces from patients in Gambia and England (13, 21); thus, the fecal-oral route of transmission seems feasible.

Houseflies (*Musca domestica*) frequently come into contact with human food and excrement and have been reported to be involved in the dissemination of numerous diseases such as cholera, shigellosis, and salmonellosis (6, 9, 10). Since houseflies habitually produce and feed on excrement (10), it is possible that they act as vectors in the transmission of *H. pylori* if they carry the bacterium and contaminate human food. This is the first report of *H. pylori* colonization in houseflies.

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MATERIALS AND METHODS

Several hundred housefly pupae (Carolina Biological Supply Company, Burlington, N.C.) were disinfected in 5% phenol solution for 10 min to reduce bacterial contamination, washed in saline, dried, placed in well-ventilated aseptic buckets, and incubated at 25°C. Adult flies emerged within 3 to 4 days, were fed on a 1:1 mixture of autoclaved granulated sugar and powdered milk, and were

kept on a 16-h photoperiod. A bowl filled with autoclaved wood chips and water served as a source of water.

The houseflies were removed and divided into groups of approximately 100 flies each. They were transferred to aseptic buckets each containing either one blood agar plate with freshly grown *H. pylori* or one sterile agar plate (control). The *H. pylori* as well as control plates in the fly cages were removed after 6 h and were replaced with sterile petri dishes containing one sterile drop of brucella broth, which served as a nutrition and water source for the flies as well as a medium for bacteria released from the flies during their feeding. At intervals of 6 h, the flies were anesthetized by keeping them at 4°C for 5 min. Six houseflies from each cage were removed and three houseflies each were transferred to sterile test tubes and into neutral buffered 10% formalin.

H. pylori isolates were originally isolated from biopsy specimens from a patient with duodenal ulcer, grown on brucella agar (Difco, Detroit, Mich.) with lysed sheep blood (5% [vol/vol]; Remel, Lenexa, Kans.), 5% and incubated in a microaerophilic atmosphere at 37°C for 72 h. To determine the viability of *H. pylori* under ambient conditions (room air and 20°C), the lids of some plates were also left open for 48 h. Samples were taken every 6 h and were inoculated onto the surface of brucella agar plates with lysed sheep blood and Skirrow's supplement, and the plates were incubated under microaerophilic conditions at 37°C for up to 5 days.

Isolation of bacteria from external surfaces of houseflies. One milliliter of sterile saline (0.9%) was added to each test tube containing three flies, and the tubes were thoroughly shaken for 2 min. A loopful of the washing was inoculated onto the surface of agar plates (MacConkey's medium, blood agar, and brucella agar with lysed sheep blood and Skirrow's supplement). The plates containing MacConkey's medium and blood agar were incubated aerobically and anaerobically at 37°C for 24 h. The bacteria were identified by colonial morphology, Gram staining, and biochemical phenotype with an automated Microscan Walk-away system (Dade, Sacramento, Calif.). The selective brucella plates were incubated microaerophilically for 48 to 72 h and were regularly screened for small *H. pylori*-like colonies. Typical colonies were subcultured and were subsequently identified as *H. pylori* by their macroscopic morphology, phase-contrast microscopy, Gram staining, and urease, oxidase, and catalase activities as described previously (8).

Isolation of bacteria from alimentary tract of houseflies. After external washing, the flies were washed in reagent alcohol for 5 min to decontaminate the external surfaces and were dried. The flies were then washed with sterile saline to remove traces of alcohol, and the alimentary tract was dissected out aseptically under a dissecting microscope. The excised gut was then homogenized in 1.0 ml of sterile saline. A loopful of the resulting homogenate was then processed as described above.

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FIG. 1. Housefly sitting on a petri dish that was left for 12 h in a fly cage containing approximately 100 flies. The surface of the plate is covered with abundant released white droplets of fly excrement.

Isolation of bacteria from excreta. The surface of the removed petri dishes, which showed numerous fly droplets (vomitus and feces) (Fig. 1), was streaked with a sterile cotton applicator and was transferred to agar plates, and the plates were processed as described above.

Histological analysis. The collected flies were placed directly into neutral buffered 10% formalin and were left in fixative for at least 72 h. Paraffin sections (5 μ m) were stained with hematoxylin-eosin and Steiner silver stain. Control sections of an *H. pylori*-positive human gastric biopsy specimen were treated similarly.

RESULTS

Microbiology. Viable *H. pylori* could be isolated from the body washings for 12 h, from alimentary tracts for 30 h, and

TABLE 1. Demonstration of viability of *H. pylori* in houseflies^a

Flies and sampling time (h)	Detection of <i>H. pylori</i> ^b			
	Body	Gut	Excreta	Agar plate
Test flies				
0	—	—	—	+
6	+	+	+	+
12	+	+	+	—
18	—	+	+	—
24	—	+	+	—
30	—	+	+	—
36 and more	—	—	—	—
Control flies				
0–30	—	—	—	—
>30	—	—	—	—

^a One hundred flies were housed in cages. The flies were exposed to agar plates containing *H. pylori* or sterile agar plates (control) placed in the cage for 6 h and were then replaced with sterile petri dishes. Six flies and the petri dishes were removed from each cage at 6-h intervals and were examined for the presence of *H. pylori*. To determine the viability of *H. pylori* at ambient conditions *H. pylori* agar plates were simultaneously exposed to room air, and samples were taken at 6-h intervals for reculture.

^b —, negative; +, positive; —, overgrowth of gram-negative bacteria, rendering isolation of *H. pylori* impossible.

from the excreta droplets for up to 30 h (Table 1). After that point, despite the initial disinfection of the pupae and aseptic handling, other gram-negative bacteria from both gut and excreta overgrew the plates and eventually swamped the selective culture plates, making the isolation of *H. pylori* thereafter impossible.

The following bacteria were grown from test and control flies during the experimental period of 5 days: *Serratia marcescens*, *Providencia rettgeri*, and *Staphylococcus aureus* from the bodies; *S. marcescens*, *P. rettgeri*, *S. aureus*, *Morganella morganii*, and *Pseudomonas aeruginosa* from the gut; and *S. marcescens*, *P. rettgeri*, *Providencia stuartii*, *S. aureus*, *M. morganii*, and *P. aeruginosa* from excreta. The bacterial colonization of both test and control flies showed increasing bacterial numbers with time.

Samples taken from *H. pylori* plates exposed to room air could only be recultured when they were transferred within 6 h to a microaerophilic environment. We were not able to reculture *H. pylori* from plates exposed to room air for 12 h or longer (Table 1).

Histology. Numerous typical *Helicobacter*-like organisms along with other bacteria could be demonstrated in the gut lumen and were also found to be attached to columnar intestinal epithelial cells in flies exposed to *H. pylori* plates (Fig. 2A). In contrast, control houseflies showed no or few non-*Helicobacter*-like organisms in their intestinal tracts (Fig. 2B). For comparison, a control section of an *H. pylori*-positive human gastric biopsy specimen is shown in Fig. 2C.

DISCUSSION

Our studies show for the first time that houseflies ingest *H. pylori* and can release the organisms as viable bacteria in excreta. In addition, houseflies can carry viable *H. pylori* on their external surfaces. In contrast to the fly gut, we could not passage *H. pylori* from plates left at room air for 12 h or longer, suggesting either that *H. pylori* is replicating within the fly's gut or that the gut is capable of functioning as a medium that preserves viability.

The mechanism of transmission of *H. pylori* has been a subject of much debate. Epidemiological studies have suggested that the fecal-oral (12) and oral-oral (2) routes likely represent the major pathways. Although *H. pylori* has been detected by PCR in Peruvian drinking water (12) and survives in tap water for a few days in the laboratory as an unculturable form (3), there is no direct evidence that the bacteria survive in significant viable and infectious forms in the environment.

The role of the housefly in the transmission of pathogens and gastrointestinal diseases such as shigellosis, salmonellosis, cholera, and yaws has already been firmly established (10). The vector role of houseflies is not a surprise if one considers their life cycle. The adult housefly deposits its eggs mainly on excrement, and its larvae feed on this material and the microorganisms in that material. The adult housefly can fly as far as 20 miles from its source (18) and can freely enter houses and areas where people congregate, as well as markets, stores, and other places where human food is available. It just as freely frequents human and animal excrement alike. Since the fly can only swallow liquid food, it usually regurgitates ingested material in order to liquefy solid materials to facilitate digestion. In addition, droplets of feces may be deposited during the feeding process. This remarkable behavior of flies in which excreta is deposited (Fig. 1) may particularly contribute to their ability to spread bacterial infections. Structurally, the fly is well adapted for picking up pathogens. Its proboscis is provided with a profusion of fine hairs that readily collect envi-

FIG. 2. (A) Numerous *Helicobacter*-like organisms admixed with other bacteria in the gut of a housefly that fed on a agar plate containing *H. pylori* 12 h earlier. (B) In contrast to panel A, the gut of a control fly, not exposed to *H. pylori*, shows an empty lumen. (C) Characteristic *H. pylori* organism seen in an human gastric biopsy specimen. For all three panels, Steiner stain was used. Magnifications, $\times 184$.

ronmental detritus. Furthermore, each of the six feet of the fly is fitted with hairy structures and pads that secrete a sticky material, thus adding to its pathogen transmission potential. It is therefore not surprising that as many as 6×10^6 bacteria have been found on the exterior surface of a single feeding fly (5), and more than 100 species of pathogenic organisms have

been isolated from the digestive tract of flies (11). It has been reported that viable *H. pylori* organisms are released in feces from infected individuals (13, 21). Fox et al. (7) were able to culture *H. mustelae* from the ferret's stool only when the animals were hypochlorhydric. Many patients develop a period of hypochlorhydria or achlorhydria shortly after the acute onset of infection with *H. pylori* (16). By analogy, this suggests that during the hypochlorhydric stage of *H. pylori* infection, there may be enhanced excretion of viable bacteria in feces, thus providing an opportunity for flies to access viable organisms.

Our data indicate that flies are able to ingest *H. pylori* during feeding on contaminated material. After *H. pylori* is swallowed by a fly, it faces a challenging environment. It passes through three distinct gut regions: the foregut, consisting of the mouth, pharynx, esophagus, and crop; the midgut, with stomach; and the hindgut, comprising the ileum, colon, rectum, and anus. *H. pylori* is exposed to substantial pH changes during passage through the midgut, as has been shown in insects of the order *Diptera*, the insect order to which houseflies belong (4, 9). The fore-midgut has a pH of 6.1, the mid-midgut has a pH of 3.1, and the hind midgut has a pH of 6.8. The low pH in the mid-midgut of flies is extraordinary, since those insects are the only invertebrates to display such an acidic region in their alimentary tract (22). Furthermore, oxyntic cells have been described in this mid-midgut region of houseflies. The oxyntic cells were morphologically similar to the parietal cells from the mammalian stomach (17, 20). The low pH enhances the actions of pepsin and lysozyme, which are also found in the fly's mid-midgut (4, 17), in order to lyse most of the ingested bacteria to be used as food. Since insects preceded humans on earth by at least 400 million years (11), we speculate that *H. pylori* evolved its unique mechanisms of survival in acidic environments, such as the insect stomach, millions of years before finding a new ecological niche, the mammalian stomach.

We postulate that *H. pylori* is acquired from human excrement by the housefly, which then, while crawling on human food, contaminates it with either regurgitated material and/or feces. The food, swallowed by a susceptible individual, then deposits *H. pylori* on the gastric mucosa, thus reestablishing infection. A similar mode of transmission has been demonstrated for *Salmonella* by Greenberg (9). Greenberg exposed houseflies to the feces of a dog infected with *Salmonella* and gave the flies access to beakers filled with cornmeal. Human volunteers who ate the meal showed traces of *Salmonella* in their stools.

In the present study, houseflies were exposed to *H. pylori* under conditions that would not occur naturally. Although the initial bacterial load of these flies was decreased by washing the pupae in phenol solution, it has been shown that about 85% of such flies are still heavily colonized with bacteria at the moment of emergence from the pupal case (9, 10). In the presence of these other bacteria, *H. pylori* was still able to colonize these flies. Although it remains to be determined if flies in their natural setting can transmit *H. pylori*, we suggest that they should be considered prime suspects for the transmission of *H. pylori*, particularly in warm and developing countries, where sanitary and domestic facilities are poor. This may explain the fact that *H. pylori* infection is almost universal among adults in developing countries, the prevalence already being 50% by age 5 years (21). The easy access for flies to outside toilets or open sewers, and hence untreated sewerage, and their ability to carry *H. pylori* could explain the high prevalence of infection in the developing world. The use of closed sewage systems would break the chain of transmission. Such a transition from out-houses to indoor plumbing occurred in the United States at about the time of World War II. This shift provides a rational

explanation for the "cohort effect" that has been widely described (19). This phenomenon notes that there has been a substantial reduction in the prevalence of *H. pylori* infection in those born during or after World War II compared with the prevalence in those born before that time. The age of acquisition of infection is predominantly in childhood, and since infection is usually life long, the prevalence at a specific age reflects childhood prevalence rates for that cohort. Eastern Europe's infection levels are relatively high. For instance, 90% of those born before 1960 in Poland are infected with *H. pylori* (14). In contrast, Western Europe, such as France, has generally lower infection rates of about 50% (15). The variable use of indoor plumbing across Europe may explain these large national differences in the prevalence of *H. pylori* which otherwise have eluded a coherent explanation.

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