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Absence of *Helicobacter pylori* within the Oral Cavities of Members of a Healthy South African Community

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Our study aimed to evaluate the oral cavity as a reservoir from where *Helicobacter pylori* may be transmitted. Histology and PCR amplification were performed. Eighty-four percent of the stomach biopsies tested positive; however, *H. pylori* was not detected in dental samples, indicating the absence of *H. pylori* within the oral cavity.

It has been suggested that the oral cavity may play a role in the transmission of *Helicobacter pylori.* Increased *H. pylori* prevalence rates have been reported in Chinese immigrants who use chopsticks and in African infants whose mothers premasticate their food (4, 16). *H. pylori* has been detected in dental plaque and saliva by culture and PCR methods, with the results obtained ranging from 0 to 100% for culture and 0 to 90% for PCR (1–3, 5, 7, 9, 11, 12, 14, 15, 17, 18, 20, 21). The role of the oral cavity as a permanent reservoir for *H. pylori* infection in healthy nonhospitalized individuals is still unclear (6, 10).

Seventy-nine (25 men and 54 women; mean age, 27 years; range, 5 to 65 years) healthy individuals from a rural community in South Africa were included in this study. We previously showed that this community had a high *H. pylori* seroprevalence rate (23, 24). The ethics committee of the University of Pretoria and the Review Board of Unitas Hospital approved this study.

Cumulative dental plaque samples were obtained from the oral cavity, and biopsies were taken from two sites in the stomach (two from the antrum and two from the corpus). One biopsy specimen from each site was placed in 10% formalin, cut, and stained with hematoxylin-eosin and methylene blue. DNA was extracted from the remainder of the samples by using the QIAamp DNA Mini kit (QIAGEN, Hilden, Germany) per the manufacturer's instructions and stored at −20°C. The quality and concentration of the extracted DNA was assessed by means of a Nanodrop Spectrophotometer (A_{260}/A_{280}, 1.6 to 2.00).

A single-step PCR directed toward the urease AB gene and a heminested PCR directed toward the phosphoglucosamine mutase (*glmM*) gene of *H. pylori* were performed on dental plaque and biopsy samples (8). A third nested PCR directed toward the 860-bp DNA region of *H. pylori* was performed on dental plaque samples (21, 22). Negative and positive controls (*H. pylori* type strain Hp115.90) were included in each batch of amplifications. Amplification was performed on the GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, Calif.). To exclude the possibility of PCR amplifications being negative due to the presence of inhibitors, the single-step PCR amplifying the urease AB gene was repeated for dental plaque samples spiked with DNA isolated from the positive control.

The sensitivities, specificities, and percentages of infected individuals are represented in Table 1. Sensitivity and specificity were calculated compared to the gold standard of histology. Dental plaque samples were not collected for five individuals, and biopsy samples were not collected for one individual included in the study. Five samples were excluded due to the fact that the A_{260}/A_{280} ratio of the extracted DNA did not fall between 1.6 and 2.00. Eighty-four percent of the individuals tested positive for *H. pylori* infection by means of histology, confirming the previously reported high prevalence of infection within this community (23, 24). None of the dental plaque samples showed amplification of the urease AB gene; however, all of the dental plaque samples spiked with the positive control showed amplification.

Controversy still exists regarding the role of the oral cavity as a possible reservoir for *H. pylori* infection in healthy individuals due to the varying results obtained by different research groups throughout the world (6, 21). It has been suggested that possible reasons for the lack of uniform positive findings of *H. pylori* within the oral environment are the sample collection procedure, methodology, and type of population studied (21).

Song and coworkers recently reported finding a characteristic distribution pattern of *H. pylori* within the oral cavity (21). If *H. pylori* has a preferred oral niche, sample collection procedures could potentially give rise to false-negative results. To exclude this possibility, cumulative dental plaque samples were collected from six different sites in the mouth.

The most commonly used PCR methods for the detection of *H. pylori* in dental plaque and saliva were evaluated by Song and coworkers. They reported that the nested PCR directed toward an 860-bp fragment of *H. pylori* genomic DNA was the

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TABLE 1. *Helicobacter pylori* test results for the community

Test	No. positive/ total no. (%)	% Sensitivity	% Specificity
Histology	66/79 (84)		
Gastric PCR			
Corpus			
Urease	58/78 (74)	88	92
glmM	62/78 (80)	95	100
Antrum			
Urease	59/79 (75)	88	100
glmM	66/79 (84)	100	100

most sensitive and specific for the detection of *H. pylori* in dental plaque and saliva (22). We included three different PCR methods: (i) single-step amplification of a 113-bp fragment of the urease AB gene, (ii) heminested PCR directed toward the *glmM* gene, and (iii) nested PCR directed toward the 860-bp fragment of *H. pylori.* The heminested PCR showed greater sensitivity and specificity than the single-step PCR for the DNA extracted from biopsy samples. No positive results were obtained when these PCR methods were performed on the dental plaque samples. The amplification of the 860-bp product as described by Song and coworkers also yielded no positive results for the dental plaque samples.

Most previously reported studies which have attempted to assess the presence of *H. pylori* within the oral environment have been conducted in symptomatic, often hospitalized patients (1–3, 5, 7, 8, 9, 11, 12, 14, 15, 17, 18, 20, 21). *H. pylori* has been successfully cultured and detected by means of PCR from vomitus by various groups (13, 19). This indicates that transient colonization of the mouth with *H. pylori* may occur after vomiting. Our study was based on a healthy study population in an attempt to exclude the possibility of detecting *H. pylori* in the oral environment due to transient colonization.

From our findings we cannot exclude that the oral cavity does not act as a reservoir for the transmission of *H. pylori* within the setting of symptomatic patients or even healthy individuals from the developed world. However, this study indicates that the oral cavity does not favor prolonged colonization of *H. pylori* in a healthy black South African population with a high prevalence of infection, indicating that another mode of transmission exists in which the oral cavity is unlikely to contribute to the spread of this organism.

(This work forms the basis of the study "A population genetics pedigree perspective on the familial transmission of *Helicobacter pylori*," which was presented by S. van der Merwe at a distinguished plenary session at Digestive Disease Week, May 2004.)

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