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Adaptive Mutation and Cocolonization during *Helicobacter pylori* Infection of Gnotobiotic Piglets

NATALIA S. AKOPYANTS,[1] KATHRYN A. EATON,[2] AND DOUGLAS E. BERG[1]*

Departments of Molecular Microbiology and of Genetics, Washington University Medical School, St. Louis, Missouri 63110,[1] and Department of Veterinary Pathobiology, The Ohio State University, Columbus, Ohio 43210[2]

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Clinical isolates of *Helicobacter pylori*, the gastric pathogen implicated in gastritis, peptic ulcers, and gastric cancer in humans, are diverse in traits likely to be important for colonization and disease. Here we report studies using a gnotobiotic piglet-*H. pylori* infection model to test for host-specific adaptation and to detect cocolonization by different strains. First, an *H. pylori* strain that initially had grown only weakly in piglets was adapted to them by spontaneous mutation and selection during 12 serial passages; this resulted in an increase in yield from about 10^3 to $>10^7$ bacteria per g of mucosa. Second, piglets were fed mixtures of two different well-adapted strains and the presence of one or both strains was monitored by restriction analysis of a PCR-amplified flagellar (*flaA*) gene segment. The restriction fragment patterns from pools of bacteria indicated that both strains had colonized most piglets and that both strains were present at more than half of the individual biopsy sites, although often at unequal ratios. This suggests a microcolonial mode of growth with limited migration of bacteria between neighboring sites in the gastric mucosa. We propose that the gnotobiotic piglet-*H. pylori* infection model will be useful for testing how spontaneous mutation, selection, and DNA transfer between strains during mixed infection may each contribute to adaptation to specific hosts and the evolution of virulence of this important pathogen.

The gastric pathogen *Helicobacter pylori* establishes chronic infections in the stomachs of more than half of all humans at some time during their lives (for reviews, see references 19 and 20). Its long-term carriage is a principal cause of gastritis and peptic ulcers (25, 31, 37) and also a major risk factor for gastric carcinoma in societies where this cancer remains prevalent (6, 26, 28). Despite this strong association, most *H. pylori* infections are asymptomatic and the factors that determine whether infection leads to overt disease are not well understood. Bacterial genotype may be important, however, because *H. pylori* isolates are diverse in traits likely to affect the efficiency of colonization, vigor of growth, or severity of tissue damage and disease. For example, clinical isolates differ in the ability to produce a potent vacuolating cytotoxin (8, 9, 24, 29, 34) and a second highly antigenic protein (the *cagA* gene product) (7, 10, 11, 35), both of which have been linked with tissue damage or ulcer formation. Bacterial strains also differ in the specificity of adherence to putative host receptors, whose abundance and distribution are affected by the human host genotype (4, 23). Thus, certain individual bacterial strains may be better suited for colonization and growth in some potential human hosts than in others.

We have begun investigating host-specific adaptation and the possibility of experimental mixed infection by different *H. pylori* strains. DNA fingerprinting studies had suggested that most infected persons in developed countries harbor just a single *H. pylori* strain (2, 18, 27), in keeping with the low overall incidence of infection. A few cases of apparent multiple infection have also been noted (18, 27), although frequencies of cocolonization in populations with different overall risks of *H. pylori* infection (32) have not been well documented and competition among strains during mixed infections has not been assessed. Understanding these aspects of *H. pylori* population dynamics seemed important, especially in light of findings that many *H. pylori* strains are easily transformed by DNA from other strains, at least in the laboratory (36). Given the diversity of *H. pylori* and human populations, cocolonization could allow interstrain DNA transfer, which could speed the emergence of new strains that are better adapted or more virulent in a given human host than the initially infecting strains, and facilitate the spread of genes for resistance to clinically useful antibiotics. These considerations prompted us to begin characterizing interactions between coinfecting strains of *H. pylori* in vivo.

Our experiments test the gnotobiotic piglet-*H. pylori* infection model for studying spontaneous adaptive mutation and growth rate selection and for studying competition and coexistence between strains of different genotypes. Earlier work had shown that gnotobiotic piglets can be infected with *H. pylori* of human origin and established the piglet model for studying virulence factors and host immune responses and for testing possible treatment regimens for eradicating *H. pylori* infection (12–16, 21). Infection is established within 24 h after oral feeding and persists for at least 12 weeks. Chronic gastritis, characterized by lymphoplasmacytic and lymphofollicular infiltration of the gastric mucosa, develops within 1 week and persists for at least 12 weeks. Neutrophilic inflammation is mild and transient but is exacerbated by previous parenteral vaccination (12). Some strains are unable to colonize piglets, and experimental infections by such strains are cleared within 2 days (13). Gastritis in piglets is comparable to gastritis in human children naturally infected with *H. pylori*. The present experiments address the following questions. (i) Do different *H. pylori* strains differ in the ability to proliferate in gnotobiotic piglets? (ii) Does animal passage of *H. pylori* lead to increased efficiency of growth? (iii) Can two divergent *H. pylori* strains establish a mixed infection? (iv) If so, does one strain tend to

* Corresponding author. Mailing address: Department of Molecular Microbiology, Campus Box 8230, Washington University Medical School, 4566 Scott Ave., St. Louis, MO 63110. Phone: (314) 362-2772. Fax: (314) 362-1232. Electronic mail address: BERG@BORCIM.WUSTL.EDU.

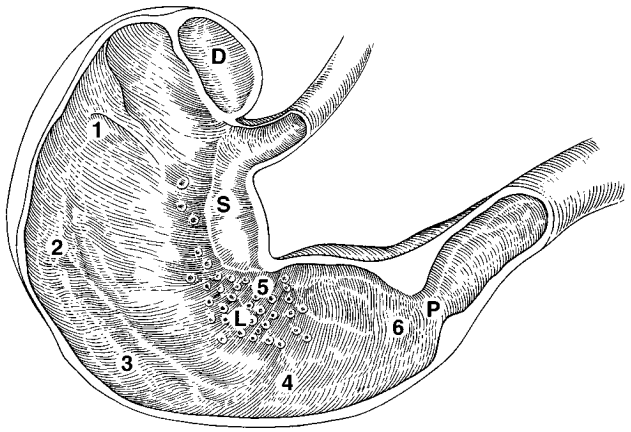


FIG. 1. Stomach of an infected gnotobiotic piglet (side view; in pigs, the greater curvature is ventral and the connections to the esophagus and the duodenum are dorsal. D, gastric diverticulum; S, squamous cardiac mucosa; P, pylorus; L, lymphoid follicles (a characteristic response to *H. pylori* infection). The numbers 1 to 6 indicate the sites at which single biopsies were taken in studies of cocolonization (see the text and Table 2 and Fig. 4).

predominate or do the strains differ in distribution among anatomic sites?

MATERIALS AND METHODS

Bacterial strains. The two strains used in this study were 26695 and WV99, both of which were originally isolated from human patients with gastritis. Strain 26695 was obtained from A. D. Pearson (15), and strain WV99 was obtained from T. U. Westblom (2). Both strains had been passaged in the laboratory prior to piglet inoculation. Strain 26695 was also adapted to gnotobiotic piglets by up to 15 serial passages before use in coinfection experiments (see below). Strains derived from such passages are designated with superscripts (e.g., 26695^{15}, to indicate the 15th-passage derivative of strain 26695). For simplicity of presentation, strains 26695^{15} and WV99 are referred to as strains A and B, respectively, in Table 2 and Fig. 4.

Each strain was rapidly motile and urease, catalase, and oxidase positive. Strain 26695 and its derivatives produced vacuolating cytotoxin, contained the cytotoxin-associated gene (*cagA*), and did not agglutinate human erythrocytes from a type O (ABO blood group) donor; in contrast, WV99 did not produce toxin, lacked *cagA*, but did agglutinate erythrocytes from the same donor (22).

Animal infections. Outbred Yorkshire cross piglets were derived by cesarian section, maintained in sterile isolators, and fed sterile sow's milk replacer (SPF-lac) as previously described (21). Bacteria used to infect them were grown in brucella broth with 10% fetal calf serum at 37°C under microaerobic conditions until the late log phase (approximately 24 h of growth; between 10^8 and 10^9 CFU/ml), harvested by centrifugation, and resuspended in sterile 1.0% peptone water at a titer of 10^9 CFU/ml. Two milliliters of this suspension was used to infect these piglets at 3 days of age by squirting the suspension into their open mouths and then allowing the normal swallowing reflex, as in previous experiments (12–16, 21). For a mixed infection, the two strains used (26695^{15} and WV99) were grown separately and mixed immediately before feeding of 10^9 CFU of each strain per piglet. Ten gnotobiotic piglets from four litters were used for mixed-infection experiments, and the piglets were sacrificed 11 to 26 days later (depending on requirements of the concomitant experiments that had financed each litter). Previous characterizations had shown that *H. pylori* infection is well established by 24 h after feeding of the bacteria to the piglets, that chronic gastritis develops within 1 week, and that the extent of bacterial growth does not vary over the following 3 to 4 weeks (12, 14–16). Conversely, *H. pylori* strains that do not colonize are cleared within 2 days and are not associated with significant inflammation (13).

At the time of sacrifice, the mucosa from half of the stomach of each piglet was removed and homogenized and serial dilutions were plated on 5% sheep blood agar plates (BBL, Cockeysville, Md.) to determine the extent of bacterial proliferation (12–14) and to sample the overall *H. pylori* population. Local *H. pylori* populations were also sampled by using one biopsy (2 by 4 mm) from each of six representative sites in the other half of the stomach: three sites from the cardiac glandular mucosa (which comprises approximately half of the piglet gastric mucosa) (sites 1, 2, and 5 in Fig. 1), two from the fundic mucosa (sites 3 and 4), and one from the pyloric antrum (site 6). Bacteria from the biopsies were spread on blood agar plates to get even distributions, and generally at least 1,000 colonies were obtained per plate. The plates were incubated at 37°C under microaerobic conditions for 3 days and harvested by scraping into brucella broth plus 15% glycerol and frozen at −70°C. Bacteria used for fingerprinting were harvested by scraping, suspended in sterile distilled water, incubated at 95°C for 5 min, and stored at −70°C until analyzed by PCR amplification and restriction endonuclease digestion of the PCR product (see below).

Histologic examination. Inflammation was quantified in gastric tissue from 24 piglets infected with strain 26695 and its derivatives. Gastric tissue was fixed by immersion in neutral buffered formalin, embedded in paraffin, cut in 6-μm sections, and stained with hematoxylin and eosin. For quantification of inflammation, surface areas of the lamina propria and lymphoid aggregates and follicles were measured with a digitizing pad and commercial software (Bioquant IV). The total surface area of lymphoid follicles or aggregates was divided by the total surface area of the lamina propria and expressed as percent inflammation (16).

Immunochemistry. Formalin-fixed, paraffin-embedded sections from 11 piglets were examined for the presence of porcine immunoglobulin A-, M-, and G-bearing plasma cells with a commercially available kit (Vectastain; Vector Laboratories, Burlingame, Calif.) as previously described (15, 21). Primary antibodies directed against porcine immunoglobulins were obtained from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, Md.).

DNA fingerprinting. Two PCR-based DNA fingerprinting methods were used. The random amplified polymorphic DNA (RAPD) method entails amplification at low stringency with oligonucleotides with arbitrarily chosen sequences to prime DNA synthesis from genomic sites that they fortuitously and usually only partially match. It results in strain-specific arrays of anonymous DNA fragments drawn from throughout the genome (38, 39) and was used to detect overall genomic divergence (or similarity) in bacteria in pure cultures. The reactions were carried out as previously described (2, 3) in 25-μl volumes containing 20 ng of phenol-extracted *H. pylori* genomic DNA; 3 mM $MgCl_2$; 20 pmol of the arbitrary primer; 1.2 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.); 250 μM each dCTP, dGTP, dATP, and dTTP (Boehringer Mannheim, Indianapolis, Ind.) in 10 mM Tris HCl (pH 8.3); and 50 mM KCl under a drop of mineral oil. A Perkin-Elmer TC480 thermal cycler was used for 45 cycles of amplification (94°C for 1 min, 36°C for 1 min, 72°C for 2 min) and then heating at 72°C for 10 min. After PCR, 8-μl aliquots were electrophoresed in 2% agarose gels containing 0.5 μg of ethidium bromide per ml and 1× Tris acetate running buffer and photographed under UV light. The 1-kb DNA ladder (Gibco-BRL, Gaithersburg, Md.) was used as a size marker in all gels. The four arbitrary primers used and their sequences were as follows: 1247, 5′-AAGAGC CCGT; 1254, 5′-CCGCAGCCAA; 1281, 5′-AACGCGCAAC; 1283, 5′-GCG ATCCCCA (1).

The alternative PCR-restriction fragment length polymorphism DNA fingerprinting method was used to characterize pools of bacteria from individual biopsies and from homogenates of large sections of the gastric mucosa from piglets infected with two bacterial strains. In this method, specific gene segments are PCR amplified with appropriate primers and digested with restriction endonucleases (1, 17). Although somewhat less sensitive than RAPD tests, this method also allows many independent *H. pylori* isolates to be distinguished from one another. It is of particular value for detecting mixed populations on the basis of the joint appearance of restriction fragments characteristic of each input strain. PCR amplification was carried out in 100-μl volumes by using 2 μl of boiled cells, 0.2 U of AmpliTaq DNA polymerase, standard PCR buffer (Perkin-Elmer Cetus), and 20 pmol of each primer (1) in a Perkin Elmer 480 thermal cycler programmed for 35 cycles as follows: 94°C for 1 min (denaturation), 50°C for 1 min (annealing), and then 72°C for 2 min (extension). After amplification, the DNA products were ethanol precipitated and resuspended in 25 μl of sterile distilled water and 3-μl aliquots were electrophoresed in a 1% agarose gel to check homogeneity and yield. PCR amplification resulted in a homogeneous DNA fragment of the size expected on the basis of the sequence. Usually, 10 μl of the product was digested with 5 to 10 U of the appropriate restriction enzyme for 1 h in the buffer recommended by the supplier (New England Biolabs, Beverly, Mass.), and electrophoresed in a gel consisting of 3.5% NuSieve GTG agarose and 1.5% Seakem LE agarose (FMC Corporation, Rockland, Maine). The primers used for PCR amplification of specific gene segments were 5′-AGGAGAATGAGATGA and 5′-ACTTTATTGGCTGGT for *ureA-ureB* (2.5 kb), 5′-TGGGACTGATGGCGTGAGGG and 5′-ATCATGACATCAGCGA AGTTAAAAATGG for *ureC-ureD* (1.7 kb), and 5′-ATGGCTTTTCAGGT CAATAC and 5′-GCTTAAGATATTTTGTTGAACG for *flaA* (1.5 kb) (1).

Figure preparation. Polaroid photographs of the gels were processed for presentation by scanning them with a Microtek Scanmaker 600ZS, using Adobe Photoshop 2.0 software (Adobe Systems, Inc., Mountain View, Calif.), and imported into Canvas 3.0.2 (Deneba Software, Miami, Fla.) running on a Macintosh Quadra 700 computer.

RESULTS

Efficiency of colonization and extent of proliferation in piglets. Strains WV99 and 26695, which had never previously infected piglets, both colonized gnotobiotic piglets, although they proliferated to very different extents. Each of the three piglets fed WV99 contained ~10^7 CFU of *H. pylori* per g of

TABLE 1. Colonization and adaptation of *H. pylori* in gnotobiotic piglets

Strain and no. of passages	No. of piglets colonized/total	Median bacterial titer[a] (CFU/g of mucosa)
WV99, 0	3/3	2.0×10^7
26695		
0	8/11	$\sim 10^{3b}$
		1×10^{5c}
2	8/8	1.5×10^5
3	13/13	4.8×10^5
10	4/4	1.1×10^6
11	18/18	1.7×10^6
12	4/4	1.5×10^7

[a] Gnotobiotic piglets were infected with 10^9 *H. pylori* cells and sacrificed after 5 to 26 days. Scrapings of gastric mucosa were homogenized, diluted, and plated for viable-count determination.
[b] Six piglets.
[c] Two piglets.

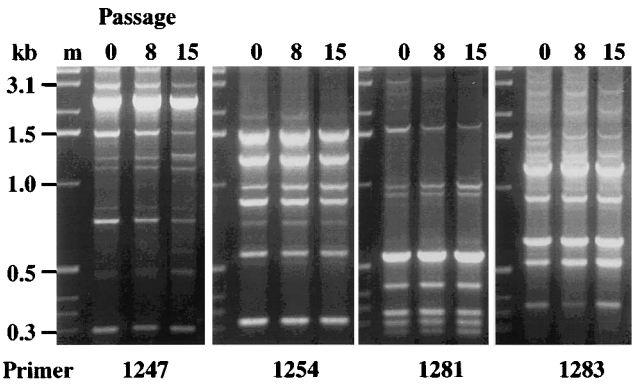


FIG. 2. Representative arbitrarily primed PCR DNA (RAPD) fingerprinting of *H. pylori* 26695 and its derivatives obtained after 8 and 15 sequential passages in gnotobiotic piglets. The faint 1.25-kb band obtained with primer 1247 only with passage 15 strain DNA was not obtained reproducibly from this DNA; similarly, the faint 1.4-kb band obtained with primer 1283 only with passage 0 and 8 strain DNAs was obtained with passage 15 strain DNA in other trials. Thus, the occasional failure to amplify these unusual nonreproducible bands does not indicate DNA sequence divergence during animal passage. Lane m contained molecular size standards.

gastric mucosa when sacrificed (5 days after infection). In contrast, only 8 of 11 piglets fed strain 26695 contained at least 10^3 *H. pylori* CFU/g of mucosa (the minimum needed for reliable detection) when sacrificed 5 to 21 days after infection; 6 of these 8 piglets contained about 10^3 CFU/g, and 2 contained 10^5 CFU/g (Table 1).

To determine if the weak growth of strain 26695 in piglets could be improved by spontaneous mutation and selection, bacteria recovered from the initial infection were amplified by growth in culture and aliquots were used to infect additional gnotobiotic piglets; this serial passage was carried out repeatedly. Table 1 shows that a derivative of 26695 that achieved about 10^4-fold higher titers in piglet stomachs emerged by the 12th such passage. Similar extents of proliferation were observed during several further passages (data not shown), although bacterial titers were not determined with precision.

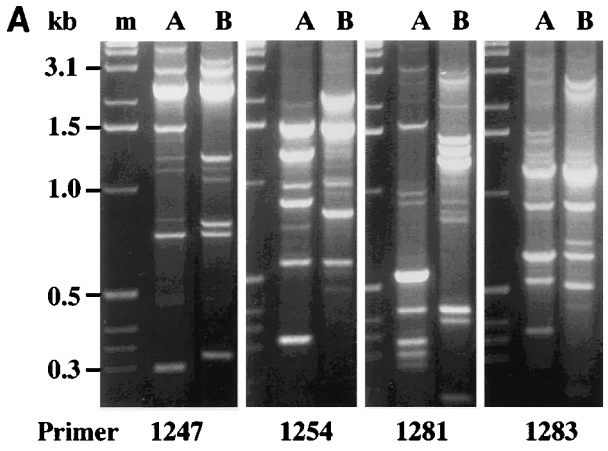
Inflammation was quantified by histological observation during the early stages of piglet adaptation. In 11 piglets infected with strain 26695^0 (strain not passaged in piglets; extent of proliferation, 10^3 to 10^5 CFU/g of mucosa), the level of inflammation (surface area of lymphoid follicles or aggregates in total lamina propria [16]) ranged from 11 to 68%. In 15 piglets infected by derivatives of 26695 that had been passaged two or three times in piglets (extent of proliferation, 10^3 to 10^6 CFU/g of mucosa), inflammation ranged from 9 to 62%. Linear regression analysis (percent inflammation versus extent of bacterial proliferation) showed a slope of -0.0035 ± 0.0150 ($r^2 = 0.0024$, $P = 0.8194$), indicating no relationship between these two variables. Similar levels of inflammation were also seen after infection by derivatives that had been further adapted by passage in piglets (extent of proliferation, $\sim 10^7$ CFU/g), although this was not quantified rigorously.

Genomic fingerprint of piglet-adapted *H. pylori*. The genomes of strain 26695 and its piglet-adapted derivatives were compared by the arbitrarily primed DNA (RAPD) fingerprinting method. As shown in Fig. 2, the arrays of fragments obtained from bacteria selected during 8 and 15 serial passages in piglets were very similar to those of the ancestral strain that had never been passaged. Since any two independent *H. pylori* isolates can usually be distinguished by such fingerprinting (2), this result verified that adaptation was due to mutation and selection, not inadvertent contamination by another *H. pylori* strain. The genomic DNAs from the passage 0, 8, and 15 derivatives of strain 26695 were also used as probes in hybridization to the sets of fragments generated by *Bgl*II digestion of each of 68 clones in an ordered cosmid miniset that covers most of the *H. pylori* genome (5). No differences were found in the hybridization patterns obtained with these probes (data not shown), suggesting that adaptation was not associated with deletion of large genomic segments. Thus, although these fingerprinting methods would not detect most simple rearrangements or point mutations, the *H. pylori* genome does not appear to be particularly destabilized by repeated shifting between growth in vitro (laboratory) and growth in vivo (piglet).

Cocolonization of piglets by two *H. pylori* strains. Strains WV99 and 26695^{15} seemed to be good candidates for mixed-infection experiments because they proliferated to similar extents during single infection in piglets (Table 1) despite several phenotypic differences (see Materials and Methods). Tests using arbitrarily primed PCR indicated that these two strains were readily distinguishable by this method (Fig. 3A). Complementary fingerprinting tests showed that the two strains also differed in restriction site patterns in each of the three different PCR-amplified gene segments tested (Fig. 3B). Such restriction site differences allowed mixed infections to be detected efficiently, as illustrated below.

To test for cocolonization, mixtures containing equal amounts of strains 26695^{15} and WV99 were fed to piglets and pools of bacteria recovered from them 5 to 26 days later were fingerprinted by *Hha*I restriction endonuclease digestion of PCR-amplified *flaA* DNA. *Hha*I-*flaA* digests were chosen because the distinctiveness and simplicity of the patterns obtained (Fig. 3B) facilitated estimation of the relative abundance of each strain in pools of bacteria (on the basis of relative yields of restriction fragments characteristic of each input strain). Tests of bacteria from throughout the gastric mucosa indicated that equal numbers of each were present in 4 of the 10 piglets tested (Fig. 4, piglet I, lane GH); strain 26695^{15} (A in Fig. 4) was more abundant than WV99 (B in Fig. 4) in another four piglets (piglet II), and only 26695^{15} was detected in the remaining two piglets (indicating that it made up more than 90% of the total) (piglet III) (Table 2).

Tests of bacteria from small biopsies (2 by 4 mm) showed that the two strains were nonuniformly distributed among sites in individual animals, even though each strain could colonize



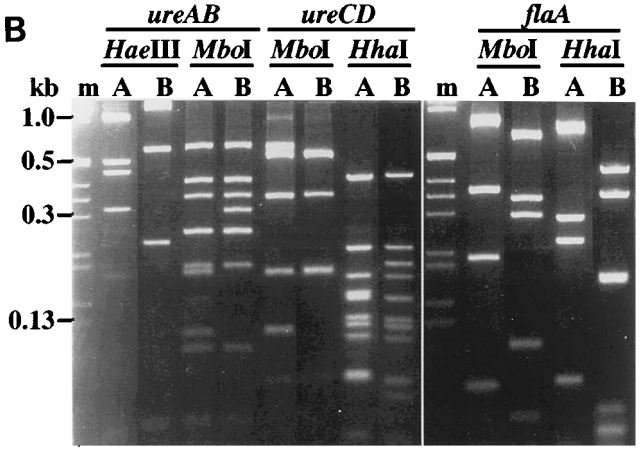


FIG. 3. DNA level comparisons of the 15th-passage derivative of strain 26695 (lanes A) and strain WV99 (lanes B). (A) RAPD fingerprinting. (B) Restriction analysis of PCR-amplified gene segments. The two strains are easily distinguished by each method. Lanes m contained molecular size standards.

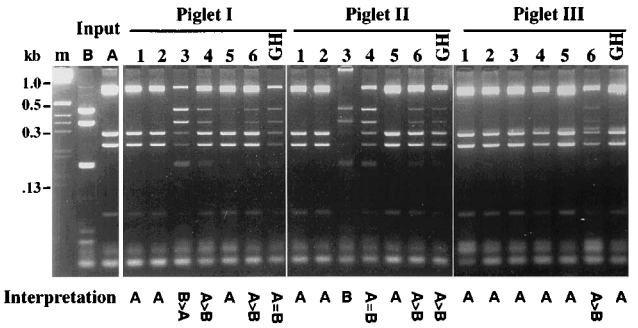


FIG. 4. Detection of cocolonization by the 15th-passage derivative of strain 26695 (A) and strain WV99 (B). Piglets I, II, and III correspond to piglets 1686, 1691, and 3086, respectively, in Table 2. The positions of sites 1 through 6 are indicated in Fig. 1. The relative abundance of each strain was inferred from the densities of characteristic restriction fragments. Lane m contained molecular size standards.

each anatomical region. For example, in piglet I (Fig. 4), which seemed to carry the same number of organisms of each strain in the stomach as a whole, only strain 26695[15] was detected at three sites (sites 1, 2, and 5); both strains were evident at the other three sites but with WV99 in excess at one site (site 3) and 26695[15] in excess at the other two sites (sites 4 and 6). In piglet II, 26695[15] was severalfold more abundant than WV99 in the stomach as a whole but only strain WV99 was detected at site 3; the two strains were equally frequent at a nearby fundus site (site 4), and only strain 26695[15] was evident at cardiac sites 1, 2, and 5. With piglet III, only strain 26695[15] was detected in the stomach as a whole but a mixed population (~20% WV99) was evident at the one antrum site biopsied (site 6).

Of the 58 biopsies from 10 piglets tested, both strains were found in 37 biopsies, only 26695[15] was detected in 20 biopsies, and only WV99 was detected in 1 biopsy (Table 2). Thus, strains WV99 and 26695[15] were found at 66 and 98%, respectively, of the sites sampled. Of 37 biopsies in which both strains were present, 26695[15] was more abundant in 17, WV99 was more abundant in 4, and the two strains were equally abundant in 16. Interestingly, strain WV99 was evident in 50% of cardiac sites, 75% of fundic sites, and 90% of antral sites, whereas 26695[15] was evident at nearly all sites throughout the stomach. Thus, despite the lack of absolute regional specificity, distal sites seemed more hospitable than proximal sites to the marginally less fit strain WV99.

DISCUSSION

We report here that (i) strains of *H. pylori* differed in the ability to grow in gnotobiotic piglets, (ii) an initially maladapted strain adapted to piglets during serial passage in them, (iii) two different strains coexisted in infected piglets, and (iv) the two strains were not uniformly distributed among biopsy sites. The observed difference among strains in the extent of growth illustrates that genetic factors can determine the extent of growth (fitness) of a bacterium in a given host; the progressive improvement of the maladapted strain with passage suggests sequential selection of several spontaneous mutations, each contributing incrementally to bacterial fitness in the piglet stomach. Since humans probably differ from one another in traits that may contribute to *H. pylori* growth (such as receptor content, immunological competence and experience, and antibiotic usage), we suggest that a strain that has become well adapted for one person might sometimes be ill suited to the next person to ingest it. Any resultant weak growth would also foster the emergence of better-adapted spontaneous mutants, essentially as documented here in piglets. In this scenario, much of the clinically relevant diversity among *H. pylori* strains today might reflect rounds of adaptation in a long succession of different human hosts.

Adaptation to piglets was not accompanied by significant changes in the arrays of RAPD products. This was expected, since each RAPD test samples only a small fraction of the target genome and mutations that do not happen to affect a primer-binding site or change the distances between pairs of such sites generally escape detection (3). The constancy of RAPD patterns during serial passage seen here and the low rates of mutation documented in other studies (36) indicate that *H. pylori* genomes are not extremely unstable. This suggests that the great diversity seen among clinical isolates (1, 2, 18, 27, 33) has another basis. Perhaps much of it reflects a long evolutionary history with no competition between strains that reside in different persons and thus only limited selection against particular genotypes. Although mixed infections might result in some interstrain competition and thus decreased genetic diversity, we suspect that such competition is less important than host-mediated selection in determining *H. pylori* population genetic structure.

TABLE 2. Frequency distribution of strains in coinfected gnotobiotic piglets

Piglet (days postinfection)	Recovery at biopsy site[a]:						
	1	2	3	4	5	6	GH
3086 (11)	A	A	A	A	A	A > B	A
3215 (11)	A	A	A = B	A > B	A > B	B > A	A > B
1685 (14)	NR[b]	NR[b]	A	A	A	A	A
1686 (14)	A	A	B > A	A > B	A > B	A > B	A = B
1691 (14)	A	A	B	A = B	A	A > B	A > B
2383 (20)	A > B	A > B	A > B	A > B	A > B	A > B	A = B
2386 (20)	A = B	A = B	A = B	A = B	A > B	B > A	A = B
2393 (20)	A	A	A > B	A	A	A > B	A > B
2396 (20)	A = B	A = B	A = B	A = B	A > B	B > A	A = B
3026 (26)	A = B	A = B	A = B	A = B	A = B	B > A	A = B

[a] Biopsy sites 1 through 6 correspond to the sites designated in Fig. 1. One biopsy was taken from each site in each animal. GH indicates homogenate of half of the gastric mucosa. A, strain 26695; B, strain WV99. Experimental infections were carried out for 11, 14, 20, or 26 days, as indicated. Note that *H. pylori* infection of gnotobiotic piglets is well established within 2 days and that bacteria are cleared from nonproductive infections within 24 h (see text). The relative abundance of the two strains in each sample was estimated by *Hha*I digestion of a PCR-amplified 1.5-kb *flaA* fragment, as illustrated in Fig. 4, on the basis of the relative abundance of restriction fragments that were characteristic of each input strain.

[b] NR, no *H. pylori* recovered.

We found that piglets could be colonized with two *H. pylori* strains when both were ingested together. Humans may be exposed to *H. pylori* throughout their lives, especially in developing countries, but it is not clear how often the simultaneous presence of more than one strain reflects sequential versus concomitant infection. Our interest in this issue was increased by finding both strains present simultaneously at many biopsy sites and by chemostat experiments indicating how balanced communities of related bacterial strains might be established and maintained (30). The nonuniformity of levels of each strain at the different biopsy sites suggests a model in which *H. pylori* grows as single cell clones (microcolonies) at many discrete foci in the gastric mucosa, with relatively little mixing, despite peristalsis and turnover of the gastric epithelium and overlying mucin. The presence of both strains in many samples suggests that these patches of growth are close to each other and/or that the bacteria migrate between different sites. In either case, the apparent ease of DNA transformation of *H. pylori* in vitro (36) suggests that cocolonization would allow interstrain gene transfer in vivo. Assuming much of the phenotypic diversity among *H. pylori* strains to be important in human infections, such gene transfer during long-term carriage in humans could facilitate host-specific adaptation, contribute to the evolution of virulence, and facilitate the spread of resistance to clinically useful antibiotics.

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