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Antimicrobial Therapies for *Helicobacter pylori* Infection in Gnotobiotic Piglets

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Gnotobiotic piglets infected with *Helicobacter pylori* were treated with various antimicrobials as monotherapy and dual therapy, and the results were compared to those for piglets treated with a triple-therapy regimen (bismuth subsalicyclate at 5.7 mg/kg of body weight, metronidazole at 4.4 mg/kg, and amoxicillin at 6.8 mg/kg four times a day [QID]). Clearance of infection was assessed after 7 days of treatment, and eradication was assessed following 7 days of treatment and a 14-day posttreatment observation interval. Monotherapy with amoxicillin, clarithromycin, and ciprofloxacin cleared and eradicated the organism from porcine stomachs; monotherapy with metronidazole cleared the infection and eradicated it from some piglets. Metronidazole-resistant microbes were recovered from treated piglets which cleared but did not eradicate the infection. Monotherapy with bismuth subsalicylate, erythromycin, nitrofurantoin, and tetracycline in the dosage range of 5.0 to 7.1 mg/kg QID was less than 100% effective in clearance and eradication, in that these drugs cleared and/or eradicated the infection from some of the piglets but did not eradicate the infection from all of the piglets. Monotherapy with an H-2 receptor antagonist (ranitidine) or a proton pump inhibitor (omeprazole) was ineffective at either clearance or eradication. In vivo dose titrations with several effective monotherapies were performed to determine the lowest effective in vivo dose of drug. In piglets, eradication was associated with a statistically significant decline in serum *H. pylori*-specific immunoglobulin M (IgM) antibodies; the titers of both IgA and IgG also declined, but the values were not statistically significant. For many antimicrobials, piglets are more sensitive indicators of clearance and eradication than humans. These data establish the *H. pylori*-infected gnotobiotic piglet as a useful model for the identification of novel antimicrobials for the treatment of this disease and for drug assessment during preclinical evaluations.

In the last 15 years, *Helicobacter pylori* infection (3, 26) has emerged as an important widespread human gastric bacterial infectious disease. Infection is acquired early in life (7, 15, 44), and although it is frequently asymptomatic, it may produce clinically apparent gastrointestinal discomfort (50). *H. pylori* is the cause of type B gastritis (50) and most instances of ulcer disease (25, 33, 35, 40) and is an important cofactor in gastric cancers (2, 5, 6, 11, 19, 36, 39, 41, 42).

Effective antimicrobial therapy for this infection presents researchers with a number of challenges (37). There is a consensus that symptomatic individuals should be treated for infection so that the more serious complications of disease can be forestalled (35). In these patients, therapeutic compliance has emerged as the most important proximate reason for treatment failure (13). Treatment of symptomatic patients in the absence of a definitive diagnosis results in unnecessary therapy for microbe-negative disorders such as nonulcer dyspepsia, heightens the risk of antimicrobial resistance (20, 34, 48), and cannot be economically justified in many instances (45).

H. pylori is susceptible to a number of different antimicrobials in vitro (14, 32) but not in vivo. Local (gastric) therapy is complicated by the short transit time of oral drugs and failure to maintain therapeutic drug levels in the stomach when drugs are administered parenterally. Monotherapy is largely ineffective (16, 17, 38, 47). Combination therapies consisting of a bismuth salt, an acid-suppressive agent, and/or one or more broad-spectrum antibiotics (12, 16, 27, 28, 38, 49) have success rates of at least 70% but have the disadvantages of complex drug interactions, unwanted side effects with one or more components, development of antimicrobial resistance (14, 20, 32, 34, 48), and compliance with a lengthy and cumbersome regimen which may last several weeks and require multiple daily dosings (12, 13, 27, 37).

Substantial progress in the understanding of the mechanisms of disease, bacterial virulence factors, and host determinants of disease have been made through parallel study of similar helicobacter infections in laboratory animals. Of these, *H. pylori*-infected gnotobiotic piglets have been shown to accurately replicate many of the features of the acute disease in humans (1, 21–24). Oral inoculation results in persistent asymptomatic colonization. The infection is multifocally distributed chiefly in the cardia and antrum. Microbes localize within the gastric mucous layer but may also adhere to epithelia; bacterial levels averaging 10^7 CFU/g of gastric mucosa are consistently obtained. Infection persists indefinitely (tested 90 days after infection and 45 days after conventionalization), in spite of strong local and systemic immunity (8, 10). Gastric lesions are characteristic and consist of diffuse to follicular lymphoplasmacytic inflammation, foci of epithelial swelling, vacuolation, necrosis, and micro- and macroulceration (1, 8, 23, 24). Chronic active (neutrophilic) inflammation occurs in piglets but is dependent upon the strain of microbe (8) and preexisting immunity (11).

The infected gnotobiotic piglet is useful for the in vivo delineation of bacterial virulence factors (8, 9). In addition, overall similarities to humans in diet (omnivores) and gastric physiology make the piglet an attractive nonprimate animal for preclinical testing of antimicrobials and other novel therapeutics. The defined microbial status of gnotobiotes and their

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environment eliminates the confounding effects of commensal microbes or their products upon antimicrobial therapies. Thus, an antimicrobial effect can be identified under the most optimal in vivo conditions. In this paper, the effects of therapy with a number of different antimicrobials upon the manifestations of *H. pylori* infection in piglets and the use of the *H. pylori*-infected piglet as an in vivo method for preclinical evaluations of antimicrobial therapy for this disease are described.

MATERIALS AND METHODS

Animals and inoculation. Procedures for derivation and maintenance of gnotobiotic piglets within sterile isolation units are described elsewhere (21). Piglets were fed a 50:50 (vol/vol) mixture of Similac with iron and sow milk replacement diet (Esbilac) three times daily. Size and nutritional requirements limit the duration of experiments with gnotobiotic piglets to 45 days or less. Isolation units were screened for microbial contaminants immediately after derivation, at least once during the experiment, and at the termination of the experiment.

Microbiology. Two- and 3-day-old piglets were orally inoculated with porcine-passaged *H. pylori* 26695 propagated to the logarithmic phase of growth in brucella broth (2.8% [wt/vol] Bacto; Difco, Detroit, Mich.) and supplemented with 10% (vol/vol) fetal calf serum as described previously (9, 10, 21, 23). For quantitative determinations of gastric bacteria, a 10% (wt/vol) homogenate of gastric mucosa in brucella broth was prepared, and duplicate 10-fold dilutions were plated onto blood agar or on chocolate agar containing trimethoprim, vancomycin, amphotericin B, and polymyxin B (Remel, Lenexa, Kans.). Plates were incubated at 37°C with 95% humidity under microaerobic (5% oxygen) conditions for 4 days prior to evaluation. Isolates were confirmed to be *H. pylori* by morphology, Gram staining, and the presence of catalase, oxidase, and urease enzyme activities.

MICs. The MICs of test agents for *H. pylori* 26695 and gastric reisolates were determined by an agar dilution method (32). Mueller-Hinton agar supplemented with 1% IsoVitaleX and 5% chocolatized sheep blood was used. The plates were incubated for 48 h at 37°C in an atmosphere of 85% nitrogen, 10% CO_2, and 5% O_2 prior to determination of bacterial growth (32).

Serologic evaluation. In some experiments, 10-fold dilutions of heat-inactivated sera were tested for isotype-specific antibodies to *H. pylori* by an enzyme-linked immunosorbent assay as described previously (9, 21).

Pathologic evaluation. Gross determinations of gastric inflammation (submucosal lymphoid follicles), mucus depletion, submucosal edema, and ulcers or erosions (if present) were made on fresh gastric tissue at necropsy. Histopathologic examination was performed with sections of formalin-fixed tissues selected from the gastric cardia, fundus, and antrum and the pyloric antrum including the torus pyloricus. Replicate sets of sections were stained with hematoxylin and eosin and the Steiner's modification of the Warthin-Starry stain for demonstration of organisms. Microscopic inflammatory lesions were scored with a semiquantitative system in which each section was assigned a numerical score of 0 if inflammatory cells were absent and the tissues were no different than the uninfected control gastric tissues, 1 if there was minimal inflammation consisting of focal collections of mononuclear cells in the lamina propria, 2 if there was moderate inflammation consisting of focal and diffuse infiltrates of mononuclear cells into the gastric lamina propria with occasional lymphoid follicles, and 3 if there was severe inflammation consisting of prominent diffuse mononuclear inflammatory cell infiltrates into the gastric lamina propria along with numerous lymphoid follicles. Epithelial changes consisted of acute cellular swelling, cytoplasmic vacuolation, and necrosis. These were variably present in all grades of severity of inflammatory lesions but were most regularly observed with the more severe (grade 3) inflammatory lesions.

Antimicrobial agents. Test antimicrobial agents were selected on the basis of published information on efficacy or lack of efficacy for the eradication of *H. pylori* in human clinical trials. Piglets were treated orally alone or in combination with the following antimicrobials: amoxicillin (Amoxil; oral suspension; SmithKline Beecham, Philadelphia, Pa.), bismuth subsalicylate (Pepto-Bismol; The Procter and Gamble Co., Cincinnati, Ohio), ciprofloxacin (Cipro IV; Miles Laboratories, West Haven, Conn.), clarithromycin (Biaxin; Abbott Laboratories, Chicago, Ill.), erythromycin ethylsuccinate (Barre-National, West Point, Pa.), metronidazole (Flagyl; Schiapparelli-Searle, Chicago, Ill.), nitrofurantoin (Furadantin; The Procter and Gamble Co., Cincinnati, Ohio), and tetracycline (Sumycin; E. R. Squibb & Sons, Princeton, N.J.). Dose and frequency varied, and these are listed in Tables 1 and 2. In addition, omeprazole (Prilosec; Merck, West Point, Pa.) and ranitidine (Zantac; Glaxo, Research Triangle Park, N.C.) were administered alone or in combination with some of the drugs. For omeprazole, beads were removed from the 10-mg capsules and were directly administered with water. For all tested antimicrobials, the dose, frequency, and duration of treatment were adjusted to be equivalent to or lower than those recommended for humans (dosage in milligrams per kilogram of body weight per day) by using the calculated average weight (1,700 g) of piglets during the treatment interval. Five piglets were treated with amoxicillin intraperitoneally twice daily (BID) for 7 days for comparison of the results with those obtained an equivalent orally administered dose of drug.

Experimental design. Two different designs were used to evaluate the antimicrobial efficacies of the test agents. Experiments were performed by litter, whose number varied from 8 to 15 piglets each. A litter was divided into two to four groups of treated piglets. For the experiments with each litter, a group of infected piglets which received either drug vehicle or brucella broth alone were included as infection controls. In the clearance design, piglets were inoculated with *H. pylori* at 2 to 3 days of age. Seven or 10 days later (9 to 13 days of age), treatment was begun and was continued daily for the next 7 days (16 to 20 days of age). The piglets were killed on the morning after the last treatment, and the effects of therapy upon gross and microscopic lesions and bacterial colonization were determined in gastric mucosal homogenates by a standard plate counting technique.

In the eradication design, piglets were infected with *H. pylori* at 3 days of age, and treatment was initiated at 10 days of age and was continued for 7 days as described above. At 17 days of age, infection status was assessed by gastric lavage. For this, 10 ml of brucella broth was introduced into the stomachs of lightly sedated piglets via a stomach tube and a syringe. Gastric contents were aspirated, tested for urease, and cultured for *H. pylori* to provide a qualitative (present or absent) assessment of gastric colonization. Twelve to 14 days after the completion of treatment the piglets were killed and the microbial status was determined by quantitative plate titrations of gastric homogenates as described above.

RESULTS

Drug efficacy. As determined by a combination of serology, pathology, and microbial culture at death, all inoculated pigs became colonized with *H. pylori*; infection was clinically asymptomatic. The results of treatment with various combination or single agent antimicrobials in both the clearance and the eradication of *H. pylori* from the infected piglets by using doses and frequencies of administration roughly equivalent to those recommended for humans are given in Table 1. A triple-therapy regimen (bismuth subsalicyclate, metronidazole, and amoxicillin), dual therapy with amoxicillin and omeprazole, and monotherapy with amoxicillin, metronidazole, clarithromycin, or ciprofloxacin cleared and eradicated *H. pylori* from the stomachs of all treated piglets. In contrast, ranitidine and omeprazole were ineffective at either the clearance or the eradication of *H. pylori*. Monotherapy with bismuth subsalicylate or erythromycin cleared and eradicated the infection from some piglets; monotherapy with nitrofurantoin or tetracycline resulted in clearance but not eradication. When the infection was not eradicated, colonization levels in treated piglets upon termination of the study were within the range of those for the positive control piglets (10^6 to 10^7 bacterial CFU/g).

By using the same eradication design, selected antimicrobials were titrated in vivo to determine the lowest effective dose (Table 1). For amoxicillin, an oral dosage of 0.9 mg/kg given four times a day (QID) achieved 100% (three of three piglets) eradication; for metronidazole, 2.5 mg/kg given QID achieved 100% (three of three piglets) eradication; dosages lower than this were not effective for some or all piglets. For clarithromycin and ciprofloxacin, dosages of 5.0 and 7.3 mg/kg QID, respectively, were the lowest dosages which achieved 100% eradication. The organisms recovered after treatment at either the clearance or the eradication endpoints were confirmed to be *H. pylori*. Homologous antimicrobial resistance of each reisolated organism to the antimicrobial used was also determined for several ineffective antimicrobials (Table 3). The MICs of metronidazole were elevated compared to those obtained for the challenge strain or isolates recovered from untreated infected piglets. An increased MIC was not observed for isolates recovered from piglets unsuccessfully treated with tetracycline, ciprofloxacin, nitrofurantoin, and clarithromycin.

By using an experimental design to evaluate the clearance of infection by killing the piglets immediately after the last treatment, additional studies with amoxicillin were performed. The combination therapy of omeprazole (5.0 mg BID) and amoxicillin (0.05 mg/kg QID) cleared the infection from all piglets

TABLE 1. Summary of efficacies of selected antimicrobial agents alone or in combination against infection of gnotobiotic piglets with *H. pylori*

Therapy and antimicrobial tested	Dosage	Clearance (no. of piglets from which infection was cleared/ total no.)[a]	Eradication (no. of piglets from which infection was eradicated/ total no.)[b]
Triple therapy			
Bismuth subsalicylate	5.7 mg/kg QID		
Metronidazole	4.4 mg/kg QID	6/6	6/6
Amoxicillin	6.8 mg/kg QID		
Dual therapy			
Omeprazole	5.0 mg BID		
Amoxicillin	5.6 mg/kg QID	6/6	4/4
Monotherapy			
Bismuth subsalicylate	5.7 mg/kg QID	5/7	2/8
Erythromycin	6.6 mg/kg QID	2/4	1/4
Omeprazole	5.0 mg BID	0/6	0/4
Ranitidine	1.6 mg/kg QID	0/4	0/4
Tetracycline	7.1 mg/kg QID	4/4	0/4
Amoxicillin	5.6 mg/kg QID	8/8	6/6
Amoxicillin	0.9 mg/kg QID	3/3	3/3
Amoxicillin	0.5 mg/kg QID	3/4	4/4
Amoxicillin	0.1 mg/kg QID	4/6	0/4
Amoxicillin	0.01 mg/kg QID	0/3	0/3
Ciprofloxacin	7.3 mg/kg QID	4/4	4/4
Ciprofloxacin	1.0 mg/kg QID	4/4	0/4
Metronidazole	4.4 mg/kg QID	5/5	5/5
Metronidazole	2.5 mg/kg QID	3/3	3/3
Metronidazole	0.5 mg/kg QID	2/4	2/4
Metronidazole	0.1 mg/kg QID	1/3	1/3
Clarithromycin	5.0 mg/kg QID	5/5	5/5
Clarithromycin	1.0 mg/kg QID	0/4	0/4
Nitrofurantoin	5.0 mg/kg QID	4/4	0/4
Nitrofurantoin	1.6 mg/kg QID	1/5	0/5
Untreated, infected controls (vehicle only or no treatment)		0/24[c]	0/25[d]

[a] Clearance was determined by microbial culture of gastric aspirates collected on the morning after the cessation of therapy.
[b] Eradication was determined by microbial culture of gastric mucosa 2 weeks after the cessation of therapy.
[c] In two additional piglets, infection was not detected by culture of gastric aspirates due to bacterial overgrowth of plates by a contaminant (*Escherichia coli*). Infection was detected in these two piglets at necropsy on day 31.
[d] One piglet died of a congenital cardiac anomaly (ventricular septal defect) unrelated to treatment between days 17 and 31 of age.

(Table 1). Amoxicillin alone, administered orally at a dosage as low as 0.05 mg/kg QID, also cleared the infection from all piglets. The same total daily oral dose of amoxicillin administered less frequently (BID versus QID) cleared the infection from some (two of five) infected piglets and reduced the level of colonization in the remaining animals. In contrast, parenterally administered amoxicillin (0.1 mg/kg BID) did not clear or suppress the infection in any (none of five) of the treated piglets.

Pathologic and serologic correlates of clearance and eradication. To determine if the pattern and severity of gastric inflammation correlated with microbial status after treatment, the histopathologic scores at each gastric sampling site of the piglets successfully treated with triple therapy or unsuccessfully with 5.67 mg of bismuth subsalicyclate per kg alone were compared to those for the untreated infected piglets (Table 4). Statistically significant differences between treatment groups by either sample site or total inflammatory score were not observed ($P > 0.60$). When these same scores were compared by microbial status as either infected (present) or eradicated (absent) at death, regardless of treatment, the mean total inflammatory score for culture-negative piglets was lower than that for the culture-positive piglets, but the difference was also not statistically significant ($P > 0.30$). In a similar fashion, mean isotype-specific antibody responses in the sera at death were compared between groups. A marginally significant ($P < 0.10$) reduction in the serum immunoglobulin M (IgM) level was detected in triple therapy-treated piglets, whereas antibody levels in bismuth subsalicylate-treated piglets did not differ significantly from those in control piglets. When these data were arranged by infection status, the trend of diminished IgM levels in successfully treated piglets ($P < 0.05$) was confirmed.

DISCUSSION

In the present study, a number of commonly used therapeutic agents were tested for antimicrobial efficacy in *H. pylori*-infected gnotobiotic piglets. As a positive control, the efficacy of triple therapy consisting of bismuth subsalicyclate, amoxicillin, and metronidazole was used as the "gold standard" of efficacy (12, 27). The oral route of drug delivery was used except in one instance (amoxicillin was given intraperitoneally), and the doses and frequencies of administration were chosen to closely replicate those used in humans. All drugs were well tolerated by the piglets, and the piglets did not have any adverse clinical effects.

Two basic experimental designs were used. The clearance

TABLE 2. Efficacy of amoxicillin upon clearance of *H. pylori* from infected gnotobiotic piglets

Antimicrobial tested	Dose (mg/kg) and frequency	Colonization in gastric mucosa	
		Clearance rate[a]	Level of colonization (mean [range] bacterial CFU/g of mucosa)
Amoxicillin and omeprazole	0.05 QID and 5.00 BID	4/4	0
Amoxicillin (oral)	0.05 QID	4/4	0
Amoxicillin (oral)	0.10 BID	2/5	—[b]
Amoxicillin (i.p.)[c] alone	0.10 BID	0/5	13.7×10^6 (3.6×10^6–29.6×10^6)
Untreated controls	None	0/9	7.2×10^6 (2.9×10^6–19.3×10^6)

[a] Number of piglets cleared of infection/total number of piglets treated.
[b] *H. pylori* was reisolated from three of five piglets, but the number of bacteria recovered was too low to be reliably quantitated.
[c] i.p., intraperitoneal administration.

TABLE 3. Susceptibilities of isolates recovered after unsuccessful treatment of gnotobiotic piglets for *H. pylori* infection

Antimicrobial	MIC (μg/ml)		
	Challenge strain (*H. pylori* 26695)	Isolates recovered after unsuccessful treatment	Isolates recovered from untreated controls
Ciprofloxacin	0.40	0.20, 0.20, 0.20	0.40, 0.40
Clarithromycin	0.80	0.80, 0.80, 0.80, 0.80	0.80, 0.80
Metronidazole	6.25	100.0, 25.0, 12.5, 100.0	6.25, 6.25
Nitrofurantoin	<0.20	<0.20, <0.20, <0.20, <0.20	<0.20, <0.20
Tetracycline	6.25	12.5, 12.5, 12.5, 12.5	6.25, 12.5

design was most useful for the rapid assessment of antimicrobial efficacy. Therapeutic efficacy was most readily determined by a combination of the eradication design and in vivo dose titration studies. The eradication design contained a 2-week posttreatment observation interval in order to more closely reproduce the pattern of treatment in clinically affected human patients. Qualitative assessment of clearance after treatment was determined by gastric lavage, in which organisms in gastric washes were detected by culture and as a result of their urease activity. While it cannot be assumed that all instances of relapse of infection after treatment would occur within this 10- to 14-day observation interval, several cases of suppression of infection followed by relapse were observed (Table 1).

In piglets, the efficacies of the combined therapy with omeprazole and amoxicillin and also the triple therapy with bismuth subsalicyclate, amoxicillin, and metronidazole are likely attributable to the antimicrobial effects of the amoxicillin component since the dose of the compound as a single agent (5.6 mg/kg) that eradicated *H. pylori* was approximately 10-fold higher than the doses eventually found to be capable of eradicating *H. pylori* from swine.

For many monotherapies, the minimal effective dose in piglets was substantially lower than those reported to be effective in humans. The most striking example of the discrepancy between humans and piglets is the efficacy data for amoxicillin. In humans, 500 to 1,000 mg of amoxicillin per kg per day is needed to clear *H. pylori* from the stomach even temporarily (4, 29). In piglets, the minimally effective oral dose range of 1.0 to 0.1 mg/kg is 1,000- to 10,000-fold less, and yet, a high dose delivered parenterally was ineffective. The latter can be attributed to the fact that the stomach may not possess a mechanism for the efficient translocation of drug from the blood in the vascular system to the mucosa (49). The impressive oral efficacy of amoxicillin was unexpected, and for this reason, titration experiments were conducted to identify the minimally effective doses of amoxicillin and other antimicrobials in piglets. The efficacy of a low dose of amoxicillin may be explained by the fact that there are no competing microbes in gnotobiotes or perhaps by the fact that a component of the normal diet binds, inactivates, or otherwise interferes with the biologic activity of amoxicillin. It is also possible that the total titratable amount (moles) of acid in neonatal porcine gastric juice (versus gastric pH) is substantially less than the moles of acid present in a comparable volume of either adult porcine or human gastric juice. If piglet gastric juice is relatively deficient in moles of gastric acid, then the bactericidal effect of amoxicillin (4, 30) would be magnified such that unexpectedly low doses of amoxicillin exhibit antimicrobial activity in vivo.

Monotherapy with metronidazole, clarithromycin, or ciprofloxacin cleared and eradicated infection from the piglets. Again, it appears that the effective dose for these agents is lower than those ordinarily recommended for humans. For these drugs, their success as monotherapies in piglets was unexpected on the basis of comparable data for humans (20, 31). Similarly, as occurs in humans (28, 47), bismuth subsalicylate monotherapy was moderately effective at bacterial clearance (five of seven piglets were culture negative after therapy) but ineffective at eradication (only two of eight piglets remained culture negative 2 weeks after the cessation of therapy). Thus, for these drugs, piglets are more sensitive indicators of clearance and eradication patterns than humans.

Erythromycin, tetracycline, and nitrofurantoin were ineffective in vivo, in spite of their documented antimicrobial effects in vitro. The explanation for this phenomenon is unknown, but this finding is characteristic of *H. pylori*. Differential susceptibility may be related to the level of metabolic activity of the microbe within the gastric microenvironment. Organisms in the plateau phase of growth are relatively resistant to the bactericidal effects of commonly used antimicrobials (31). It is possible that the microbes within the gastric niche are physiologically inactive and are thus relatively resistant to bactericidal agents. Alternatively, these antimicrobials might not reach the gastric mucosa in sufficient quantities or may not remain there

TABLE 4. Correlation between histopathologic and serologic results and efficacy of therapy in eradicating *H. pylori* from the gastric mucosa of infected gnotobiotic piglets

Treatment group or infection status	No. of piglets	Gastric inflammatory score[a]	Serology result[b]		
			IgG	IgM	IgA
Treatment					
None (vehicle controls)	3	5.3 ± 3.2	0.75 ± 0.26	0.57 ± 0.10	0.57 ± 0.39
Triple therapy[c]	6	4.7 ± 2.7 (NS)[d]	0.75 ± 0.16	0.39 ± 0.14	0.29 ± 0.12
Bismuth subsalicylate[e]	8	4.3 ± 1.8 (NS)	0.88 ± 0.24	0.61 ± 0.15	0.40 ± 0.29
Status of infection (regardless of treatment)					
Present	9	5.2 ± 1.9	0.90 ± 0.27	0.64 ± 0.10	0.50 ± 0.31
Absent	8	4.4 ± 2.3 (NS)	0.71 ± 0.16	0.40 ± 0.12[f]	0.26 ± 0.12

[a] Sum of inflammatory scores (0 to 3; total of 12 possible) from the four regions (cardia, fundus, antrum, and pylorus) examined. Histopathologic data are expressed as the mean ± standard deviation score.
[b] Serologic data are expressed as mean ± standard deviation optical density at 450 nm.
[c] Triple therapy consisted of bismuth subsalicylate (5.7 mg/kg QID), metronidazole (4.4 mg/kg QID), and amoxicillin (6.8 mg/kg QID).
[d] NS, not statistically significant.
[e] Bismuth subsalicylate (5.7 mg/kg QID) was used alone.
[f] Statistically significant (versus presence of infection) with a P value of <0.05.

for a sufficient length of time to exert effective antibacterial activity in vivo (49).

In general, nonmicrobial antemortem indicators of infection were weakly correlated with posttreatment antimicrobial status, a finding likely attributable to the brief posttreatment observation interval of 2 weeks or less. However, as a group, infected piglets could be distinguished from successfully treated piglets by determination of the levels of antibodies of the IgM isotype class in serum by enzyme-linked immunosorbent assay. This is consistent with our previous work which showed that explants prepared from the gastric mucosa of infected piglets rapidly cease local immunoglobulin production (24) following the successful eradication of microbes, even though serum antibody levels remain elevated. It is likely that both IgG and IgA antibody levels would have decreased to undetectable levels over time if the posttreatment observation interval were longer. Like antibody responses, local manifestations of gastric inflammation also regress after successful antimicrobial therapy, although the semiquantitative scores were not statistically significantly reduced in this study. Thus, piglets are similar to humans, in that neutrophilic infiltrates disappear by 6 weeks or earlier after the cessation of treatment; lymphocytic infiltrates also regress but may take several months to return to the normal levels in the gastric mucosa (50). In piglets, microbial culture and reisolation is a more reliable method than either serology or histopathology for determining efficacy.

In humans, the development of microbial resistance after failed therapy or infection with resistant microbes in the absence of a known previous exposure to them is a known complication of therapy with clarithromycin and metronidazole (14, 20). It is estimated that 7% of *H. pylori* isolates are resistant to clarithromycin (20) and that up to 54% are metronidazole resistant (34). Strains of *H. pylori* resistant to ciprofloxacin, metronidazole, and erythromycin can be produced in vitro by rapid passage in the presence of these antibiotics (14). Our strain (strain 26695) was uniformly susceptible to all antimicrobials in vitro. Only in metronidazole-treated piglets did resistant strains develop; these were recovered and resistance was confirmed by the substantially elevated MICs determined in antibiotic sensitivity tests performed with the reisolated organisms. Here, the gnotobiotic conditions of the experiment exclude the possibility that the recovered strains acquired resistance by plasmids or other genetic exchange mechanisms which may occur between colonizing microbes and commensal organisms. It thus appears that for this drug at least, the development of resistance in vivo is an inducible event.

Drugs which inhibit gastric acid production either as H-2 antagonists or as proton pump inhibitors were uniformly unsuccessful in clearing or eradicating *H. pylori* from infected piglets. These findings reflect similar data for humans (18, 43, 46, 47) and indicate that these drugs as monotherapies for the clinical relief of gastric hyperacidity or gastritis exert their beneficial effects without significantly reducing the levels of bacterial colonization in the stomach. As indicated above, the apparent efficacy of combined therapy with omeprazole and amoxicillin in piglets and perhaps humans is likely attributable to the antimicrobial effects of amoxicillin alone.

In infected humans, patient compliance is a major risk factor for therapeutic efficacy (13, 37). In gnotobiotic piglets, compliance can be rigorously controlled. A prominent advantage of piglets as a preclinical treatment trial model is the fact that piglets appear to be remarkably sensitive to antimicrobial therapies for this infection. Because of this, it is reasonable to conclude that if a novel antimicrobial agent or therapeutic approach does not affect colonization levels in piglets, the likelihood of success as a monotherapy when applied to humans is low. Like humans, serologic and histopathologic correlates of infection appear to resolve following eradication of the infection. Gastric physiology and anatomy in piglets also closely resemble those in humans. Thus, the infected gnotobiotic piglet represents an excellent screening model for eliminating ineffective therapies and can be used to rank order the efficacies of single agents by differentiating those which are more effective (e.g., amoxicillin and metronidazole) from those which are less effective (e.g., ciprofloxacin and clarithromycin) and ineffective (e.g., nitrofurantoin and ranitidine). Finally, the model has the potential to predict issues associated with the in vivo development of antimicrobial resistance and possibly unwanted interactions between drugs in combination therapies in an environment devoid of microbial competition or other outside influences. Thus, the porcine model of infection with *H. pylori* is useful for evaluating the efficacies of test agents for therapy of infection in humans.

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