INFECTION AND IMMUNITY, Nov. 1987, p. 2789–2796
0019-9567/87/112789-08$02.00/0

Vol. 55, No. 11

Establishment of Gastric *Campylobacter pylori* Infection in the Neonatal Gnotobiotic Piglet

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Received 16 June 1987/Accepted 8 August 1987

***Campylobacter pylori*, a gram-negative microaerophilic bacterium, has been implicated in the genesis of human gastritis, dyspepsia, and gastroduodenal ulceration. Previous attempts to reproduce the diseases in conventional laboratory animal species have been unsuccessful. To determine if neonatal gnotobiotic piglets were susceptible to *C. pylori*, we orally challenged two litters ($n = 17$) with 10^9 CFU after pretreating them with cimetidine. Controls housed in separate units received nothing or peptone water alone. Piglets were examined 1, 2, 3, and 4 weeks after challenge. Colonization by the bacterium and inflammation of the gastric mucosa persisted throughout the study period. Organisms were revealed by Warthin-Starry silver stain to reside between the mucus layer and the gastric epithelium. Culturing of samples from sites along the gastrointestinal tract revealed that the bacterium colonized essentially only the gastric and proximal duodenal mucosae. Gross pathological changes were restricted to the stomachs of infected piglets and consisted of submucosal edema, increased gastric mucus production, and progressive development of mucosal lymphoid follicles. Microscopic lesions consisted of transient neutrophilic infiltrates followed by diffuse and follicular infiltrations of mononuclear leukocytes into the mucosa and submucosa. Alcian blue–periodic acid-Schiff stains suggested that the infection resulted in the depletion of mucopolysaccharide production by deep gastric glands. These data indicate that gnotobiotic piglets reproduce many of the features of diseases associated with *C. pylori* in humans.**

Chronic gastritis and attendant clinicopathologic entities, such as nonulcerous dyspepsia and gastroduodenal ulceration, are commonly encountered in human gastroenterology (3, 6). The etiology and pathogenesis of these conditions are unclear but have been linked to living conditions of high stress characteristic of western society, diet, and possibly duodenogastric bile and acid reflux. Symptoms include indigestion, sternal or epigastric discomfort, generalized abdominal pain, burping, gastric distention, and halitosis (6). Numerous nonprescription drugs, chiefly antacids and antifoaming agents, are available, and affected individuals are encouraged by media advertising to indulge in self-treatment regimens with these products.

An emerging candidate agent for the genesis of gastritis or ulceration is a gram-negative spiral bacillus, *Campylobacter pylori*, first cultured from gastric mucosa in 1984 (17). Previous workers noted the occurrence of "spirochetes" in gastric tissues, but disease associations were not made (for a review, see reference 6). Since that initial report from Australia, a number of investigators have reported similar findings (2, 11, 21–24), although not all are in agreement as to their pathologic significance (8, 14). In attempting to link isolations to disease processes, most authors emphasize the importance of concurrent histopathologic examination of gastric biopsies (6, 15). When these studies are performed carefully with the understanding that gastritis associated with *C. pylori* is a focal or multifocal lesion, the correlation of lesions with successful bacterial isolations is high (1, 6, 15).

Prospective clinical, pathologic, and immunologic investigations are needed to confirm or deny the role of *C. pylori* in human gastritis. Clinical investigations into naturally occurring diseases in humans have the disadvantages of patient selection, development of inclusive (and exclusive) diagnostic criteria, design of a therapeutic regimen(s), and compliance with prescribed treatment. Oral infection of human volunteers obviates many of these problems but brings with it expense and limited flexibility in the design and execution of microbiologic and pathologic studies. What is clearly needed is an animal model system in which disease factors can be easily manipulated and the limitations inherent in the use of experimental human subjects can be avoided.

To this end, oral challenge of numerous adult and neonatal laboratory animal species, including mice, rats, rabbits, guinea pigs, and germfree rats, has been performed (6; D. R. Morgan, unpublished data); all have been unsuccessful. All of these species have the disadvantage of a gastrointestinal system notably different from that of humans. The pig is a functional monogastric mammal with dietary habits and anatomical and physiological characteristics similar to those of humans (9, 12). Thus, the primary objective of this study was to determine if gnotobiotic piglets are susceptible to oral challenge with *C. pylori*. Specifically, we wished to determine whether gastric infection is established and what the microbiologic and pathologic consequences of gastritis induced by *C. pylori* are.

(This work was presented in part at the annual meeting of the Infectious Disease Society of America in New Orleans, La., on 3 October 1986.)

MATERIALS AND METHODS

Animals. A total of 17 gnotobiotic domestic Yorkshire piglets from two litters (8 and 9 per litter) were used in this study. Both litters were derived from date-mated pregnant sows by Caesarian section essentially by procedures described previously (25). Neonatal piglets were transferred into sterile pentub isolation units containing six partitions, an exterior heat source was applied, and the piglets were fed

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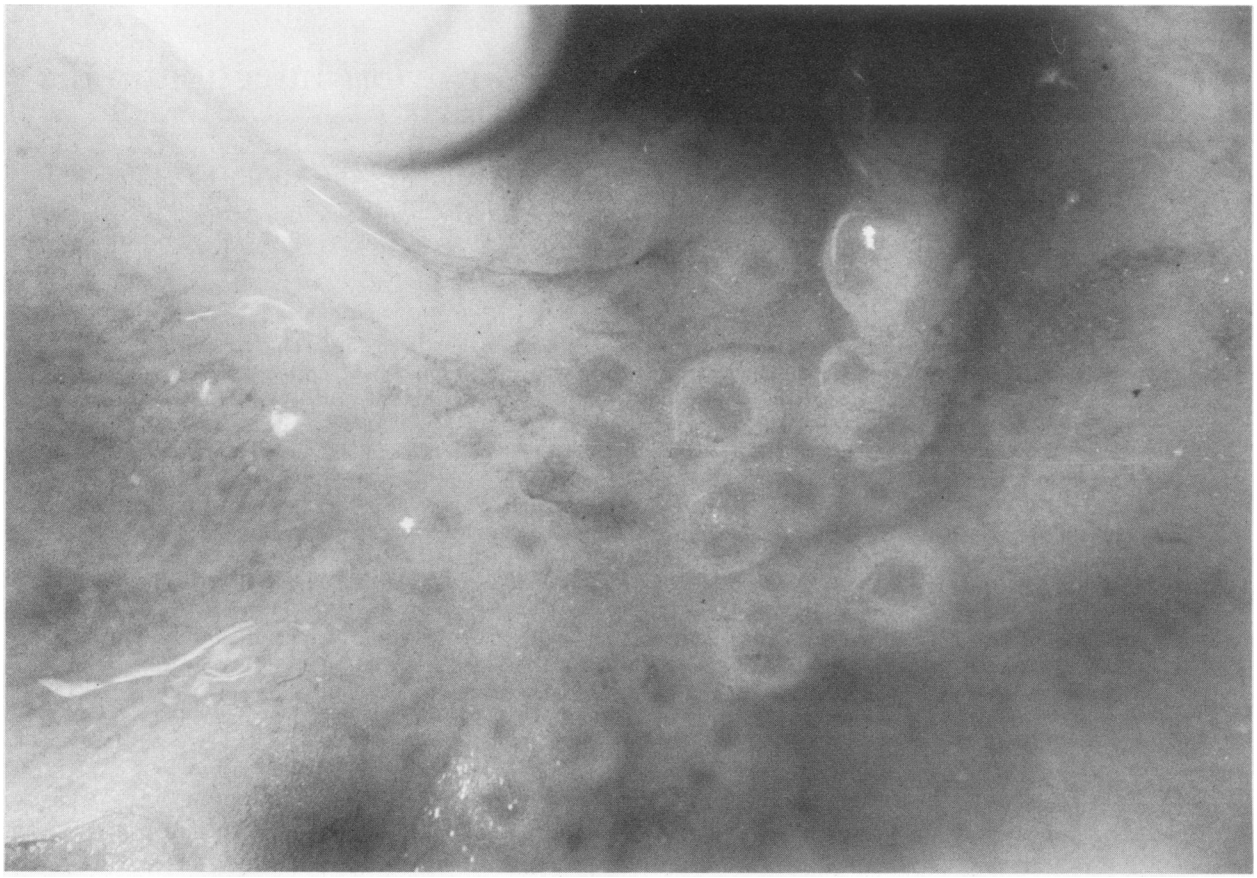

FIG. 1. Submucosal lymphoid follicles in the mucosa and submucosa of a piglet challenged orally with *C. pylori* 32 days previously.

a diet of Similac plus iron per os three times daily (100 to 150 ml per feeding). Uninfected controls were maintained in separate isolation units.

Preparation of bacterial inoculum. Forty-eight-hour broth cultures of *C. pylori* were used to prepare the challenge inoculum. Bacteria were grown in brucella broth (Difco Laboratories, Detroit, Mich.) supplemented with 10% fetal bovine serum and 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) in 50-ml Erlenmeyer flasks. Flasks were placed in GasPak (BBL Microbiology Systems) jars with a CampyPak (BBL Microbiology Systems) to provide an appropriate atmosphere, and the jars were shaken on a gyratory platform (120 rpm) at 37°C. Cultures were harvested by centrifugation, suspended in peptone water, and enumerated by standard plate counting before challenge.

Microbiology. Daily fecal swabs for bacterial cultures were taken from all piglets in litter 1. Swab samples were taken from the surfaces of the cardium, fundus, pylorus, and duodenal bulb in experiment 1. In experiment 2, swab samples were taken from the stomach regions, oropharynx, esophagus, duodenum, jejunum, ileum, spiral colon, terminal colon, and rectum. Samples were inoculated onto GCHI agar plates supplemented with trimethoprim, vancomycin, polymyxin B sulfate, and amphotericin B (Remel, Lenexa, Kans.) for bacterial isolation. The plates were incubated at 37°C in a GasPak jar for 3 to 7 days. Isolates were identified as *C. pylori* on the basis of Gram stain reaction and oxidase, catalase, and urease production.

Experimental design. At 4 and 5 days of age, piglets were rendered temporarily achlorhydric by oral administration of cimetidine (60 mg/kg). Infected piglets received a suspension of *C. pylori* containing 10^9 CFU in 2.0 ml of peptone water orally on day 5 of age after a 12-h fast. Controls received no treatment (litter 1) or peptone water alone (litter 2).

The infection studies were conducted by litter in two experiments. Piglets were examined three times daily for clinical signs of disease. In experiment 1 (eight piglets), six piglets were challenged with 3.8×10^9 CFU of *C. pylori* and two piglets were used as controls. Piglets were euthanized and examined 1 (two infected and one control), 2 (two infected), and 3 (two infected and one control) weeks after challenge. At necropsy, the stomachs were opened along the longitudinal axis, and swab and tissue samples were collected from the above-mentioned four anatomical regions. Gross lesions, when present, were noted.

In experiment 2 (nine piglets), six piglets were challenged with 4.4×10^9 CFU of *C. pylori* as described above and three piglets were used as negative controls. Piglets were euthanized and examined 1, 2, and 4 weeks after challenge (two infected and one control at each interval).

Histopathology. Tissue samples for histopathology were taken from sites adjacent to those sampled for microbiology. In experiment 1, samples were fixed in 10% neutral buffered Formalin. In experiment 2, the stomachs of one infected piglet and the uninfected control piglet from each interval were removed by ligation and resection and inflated with a fixative composed of 4% (vol/vol) Formalin and 1% (vol/vol) glutaraldehyde in a phosphate buffer (4F1G) formulation (18). After fixation, tissue samples were embedded in paraffin and sectioned as 6-μm pieces, and replicates were

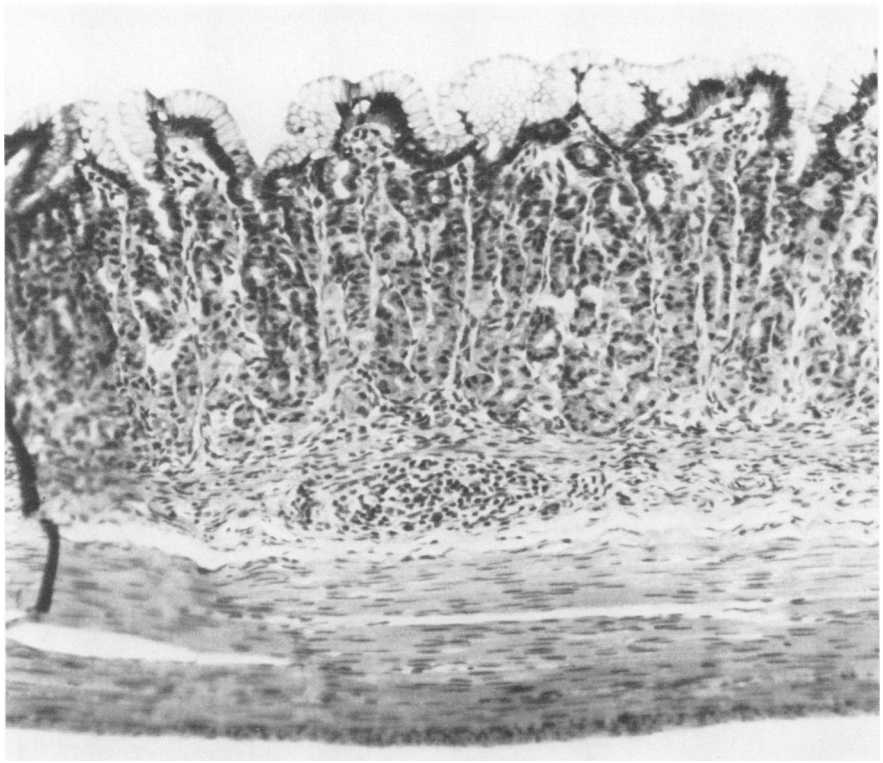

FIG. 2. Rare small aggregate of lymphoid cell elements in the lamina propria of the pylorus in an uninfected control piglet. Hematoxylin-eosin stain.

stained with hematoxylin-eosin, Warthin-Starry (WS) silver, and Alcian blue–periodic acid-Schiff (PAS) stains.

Serology. At necropsy, a terminal serum sample was collected from each anesthetized piglet and frozen for the subsequent determination of *C. pylori* antibody. Sera were tested for the development of antibody by a standard enzyme-linked immunosorbent assay. Formalinized broth-cultured *C. pylori* (10^7 CFU per well) was used as the antigen in microtiter plates. Alkaline phosphatase-conjugated affinity-purified goat anti-swine immunoglobulin G (Bio-Rad Laboratories, Rockville Centre, N.Y.) was used as the secondary reagent for pig antibody.

RESULTS

Clinical signs and macroscopic lesions. Infection did not result in grossly visible gastric epithelial erosions or ulcerations. An increase in luminal gastric mucus in infected versus control piglets was especially prominent from 2 weeks after challenge on. Other tissues, including the intestine, were unremarkable. In experiment 1, four of six infected piglets exhibited mild transient diarrhea 2 days after challenge; one piglet was listless and mildly anorectic 2 to 4 days after challenge. In experiment 2, similar results were obtained; several infected piglets exhibited transient anorexia. Prominent submucosal and mucosal lymphoid follicles (nodules) were seen in both infected piglets sacrificed 4 weeks after challenge (Fig. 1).

Microscopic lesions. Histopathologic lesions indicative of chronic active gastritis were noted in all piglets infected with *C. pylori*. The intensity and severity of these changes increased with time. For convenience, lesions detected in both experiments are described together. Microscopic changes in uninfected controls were quantitatively similar throughout and consisted of occasional mononuclear cells found in the submucosa of the gastric fundus and pylorus. Rare small submucosal lymphoid aggregates were seen (Fig. 2). Neutrophils were never observed.

One week after challenge with *C. pylori*, gastric cardiac regions contained neutrophilic infiltrates largely restricted to the nonglandular (i.e., epithelial) regions of the cardia. Neutrophils formed intraepithelial aggregates (microabscesses) and were also present in the lamina propria. Mononuclear leukocytes were also detected in this region but were more prominent in the nonglandular regions. Aggregates of cells were present in the submucosal regions and occasionally obliterated crypt regions. Small lymphoid follicles were detected in the cardia.

Two weeks after challenge, the neutrophilic response had resolved. There was a notable increase in the number of mononuclear cells in both the submucosa and the lamina propria. In the latter regions, discrete lymphofollicular aggregates were apparent. Three weeks after challenge, the microscopic lesions had intensified, chiefly because of the infiltration and proliferation of mononuclear cells. Lymphoid follicles were prominent and occasionally coalesced to form large sheets of cells encompassing both the submucosa and the lamina propria (Fig. 3).

The Alcian blue-PAS histochemical stain is useful in determining the ability of gastric epithelia to secrete various general classes of mucopolysaccharides. A deep-blue-staining product (Alcian blue) is considered indicative of acid mucopolysaccharide (AMP) production, whereas a PAS-positive reaction product (red) stains predominantly neutral mucopolysaccharides (NMPs). Each of the four anatomical regions of the stomach was evaluated for types

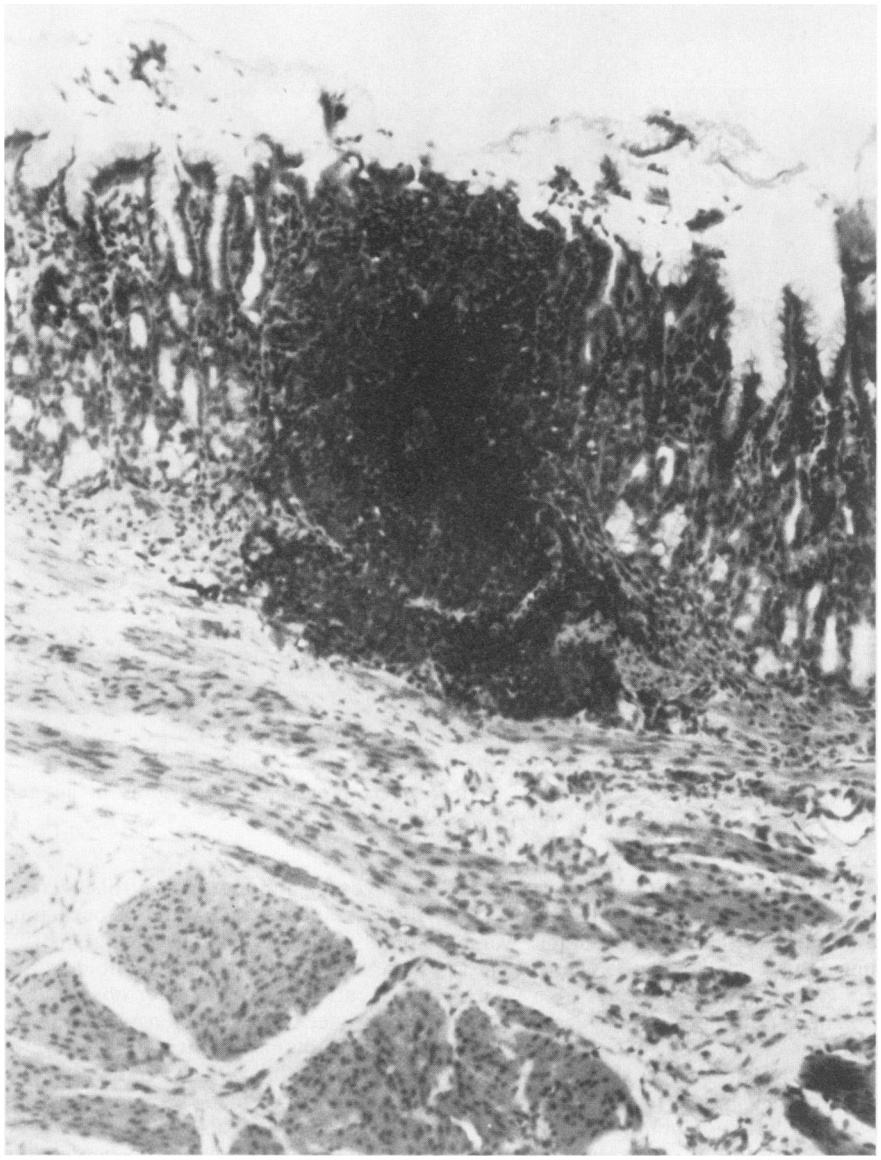

FIG. 3. Lymphoid follicle development in the submucosa and lamina propria of a gnotobiotic piglet 20 days after challenge with *C. pylori*. Hematoxylin-eosin stain.

and ratios of stained cells at the luminal (i.e., superficial gastric mucus-secreting epithelium) and deep glandular (mucus-secreting cell) pits. Tables 1 and 2 summarize these findings.

Controls showed essentially the same pattern of reaction over time. At the luminal epithelial surface, AMP production predominated in the cranial (cardia and fundus) regions, whereas the frequency and intensity of NMP production increased in the pylorus and duodenal bulb. In gastric pits, the opposite reaction pattern was observed. That is, in the cardia and fundus, NMP production predominated, whereas in the distal regions, AMP production predominated.

In infected piglets, there was a reduction in AMP-positive luminal cells in the anterior regions of the stomach in the later stages (2 and 4 weeks after challenge) of infection, as compared with controls; distal staining patterns remained unchanged and were indistinguishable from control staining patterns. Staining patterns in gastric glands in infected piglets were indistinguishable from those in controls 1 week after challenge. However, 2 and 4 weeks after challenge, there was a marked reduction in and depletion of both AMP and NMP production by deep gastric glands in sections of the fundus and pylorus. These regions correspond to those in which histopathologic lesions associated with *C. pylori* infection were most prominent.

Microbiology. Table 3 summarizes the results of both WS silver staining and bacterial isolation from the stomachs of infected and control piglets in both experiments. Organisms were recovered from at least one anatomical region from all infected piglets at each examination interval after challenge. In experiment 2, organisms were observed in or recovered from the esophagus of two piglets (1 and 4 weeks after challenge) and in the duodenum of two piglets (2 and 4 weeks after challenge). In these instances, however, the tissues did not exhibit the histopathologic lesions observed in the stomach, and it is most likely that these isolation data reflect

TABLE 1. Alcian blue-PAS staining patterns in sections of gastric mucosa from the three uninfected control gnotobiotic piglets of litter 2

Section and piglet no.	Cell ratio[a] in indicated region:			
	Anterior		Distal	
	Cardia	Fundus	Pylorus	Duodenal bulb
Luminal surfaces				
86-3229	10:1	4:1	1:10	1:10
86-3227	4:1	1:4	1:10	1:10
86-3228	10:1	1:4	1:10	1:10
Gastric glands				
86-3229	1:10	1:10	1:1	4:1
86-3227	1:10	1:10	1:1	10:1
86-3228	1:10	1:10	1:1	10:1

[a] Data are expressed as the ratio of Alcian blue (AMP)-positive to PAS (NMP)-positive mucus-secreting cells on luminal surfaces and in gastric glands.

spillover from the stomach which occurred during the manipulations performed for gastric ligation and resection. Organisms were not recovered from the feces.

Organisms were not revealed in controls by WS silver staining. In contrast, organisms were revealed by WS silver staining in all infected animals in at least one of the four anatomical regions throughout the 4-week study period. Bacteria were largely restricted to the superficial mucus-secreting layer of the gastric epithelium (Fig. 4). Organisms were extraepithelial in location and appeared to be attached to the glycocalyx of the cells beneath the acellular mucus layer. Occasionally, organisms were noted in the deeper portions of the mucosa. As before, organisms appeared to be extracellular and were restricted to the lumina of occasional dilated gastric pits. Structurally intact organisms were not seen in the submucosa or lamina propria.

Serology. Specific antibody to *C. pylori* was present in every challenged animal from 2 weeks after challenge on (Table 4). Animals sacrificed 1 week after challenge and all control animals, irrespective of the time of sacrifice, had no serum antibody specific for *C. pylori*.

DISCUSSION

The data reported in these experiments demonstrated that neonatal gnotobiotic piglets were susceptible to oral challenge and subsequent gastric colonization by *C. pylori*. Infection appeared to be largely restricted to the stomach and persisted for longer than 4 weeks after oral challenge. The organism was not shed in a viable form in the feces and did not appear to colonize other, nongastric segments of the intestinal tract. Infected piglets were largely asymptomatic. Macroscopic lesions, if present, were subtle, correlating well with human gastritis associated with *C. pylori*. Gastric infection resulted in the development of characteristic microscopic lesions which were first detected as a transient and inconsistent neutrophilic infiltration into the nonglandular cardia. Subsequent lymphocytic gastritis of the glandular portion of the stomach progressed from focal accumulations of cells in the submucosa and lamina propria to the formation of discrete lymphoid follicles located in both the submucosa and lamina propria. The WS silver stain was useful in identifying the site(s) of colonization within the stomach. Most of the organisms were located extracellularly on the superficial (luminal) epithelial surface between the epithelial cells and the superficial protective mucus layer. This tropism corresponds to similar sites of colonization in humans (6, 7, 15). When compared with controls, piglets infected with *C. pylori* exhibited histochemical changes in mucopolysaccharide production by gastric mucus-secreting epithelium. Grossly, this was manifested as an apparent increase in free mucus in the stomach. Histologically, this was manifested chiefly as a reduction in the amounts of mucopolysaccharides in the deep gastric glands, which correlates with the depletion of the mucus layer associated with human gastritis (5). The Alcian blue-PAS stain is not quantitative and is useful only in identifying trends. Clearly, more precise biochemical measures of these changes are needed before definitive statements regarding the effects of infection upon mucus production can be made.

The pattern of histologic lesions observed was remarkably similar to that observed in humans with gastritis associated with *C. pylori* (6, 15, 23). Others have emphasized neutrophilic infiltrates in affected human gastric mucosa (6, 15). In piglets, neutrophilic responses were transient, inconsistent, and largely restricted to the nonglandular cardia. A consistent finding in humans is a reduced mucus content in mucosal cells (6). Similar changes were apparent in tissue sections examined 4 weeks after challenge. It is likely that further reductions in mucus production would have occurred with time, thereby ultimately mimicking human lesions. This phenomenon in piglets remains to be determined, however.

The development of specific antibody to *C. pylori* after challenge is consistent with a true infection by this bacterium. Humans with gastric *C. pylori* infections produce specific antibody and, in this way, the porcine model parallels human disease. Serum antibody may be a useful diagnostic indicator of human *C. pylori* infections (1, 8, 10, 16).

Except for transient intraepithelial abscesses and attendant microscopic epithelial erosions in the nonglandular (cardiac) portion of the stomach, ulceration of the gastric

TABLE 2. Alcian blue-PAS staining patterns in sections of gastric mucosa from the gnotobiotic piglets of litter 2 infected with *C. pylori*

Section, day postinfection, and piglet no.	Cell ratio[a] in indicated region:			
	Anterior		Distal	
	Cardia	Fundus	Pylorus	Duodenal bulb
Luminal surfaces				
7				
86-3221	10:1[a]	4:1	1:10	1:10
86-3222	10:1	4:1	1:10	1:10
14				
86-3223	4:1	1:1	1:10	1:10
86-3224	4:1	4:1	1:10	1:10
32				
86-3225	10:1	1:1	1:10	1:10
86-3226	1:10	0[b]	1:10	1:10
Gastric glands				
7				
86-3221	1:5	1:5	1:1	4:1
86-3222	1:10	1:10	1:1	10:1
14				
86-3223	1:10	—[c]	—	3:1
86-3224	1:10	—	—	3:1
32				
86-3225	1:10	—	1:10	4:1
86-3226	1:10	1:10	1:1	10:1

[a] See Table 1, footnote *a*.
[b] 0, A relevant area(s) of the mucosa on the microslide was missing.
[c] —, Depletion (i.e., a complete lack of an AMP or NMP reaction product).

TABLE 3. Microbiological findings in the gastric anatomical regions of gnotobiotic piglets infected with *C. pylori*

Piglet group and time after challenge	No. of piglets from which a viable culture was obtained/total no. examined for indicated region:				No. of piglets in which organisms were revealed by WS silver staining/total no. examined for indicated region:			
	Cardia	Fundus	Pylorus	Duodenal bulb	Cardia	Fundus	Pylorus	Duodenal bulb
Uninfected controls (n = 5)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Infected piglets								
1 wk (n = 4)	2/3	2/3	2/3	2/3	3/4	3/4	3/4	3/4
2 wk (n = 4)	3/3	2/3	3/3	2/3	4/4	2/4	2/4	3/4
3 wk (n = 2)	2/2	2/2	2/2	2/2	2/2	1/2	2/2	1/2
4 wk (n = 2)	1/1	1/1	1/1	1/1	0/2	2/2	2/2	2/2

glandular mucosa in infected piglets was not observed. Among domestic animal species, pigs are noted for the spontaneous development of gastric ulceration and perforation (4, 9, 19, 20). In a sense, this syndrome is incorrectly named, since the ulcers are usually restricted to the nonglandular (esophageal or cardiac) region of the organ. Although the pathogenesis is unknown, naturally occurring ulceration is seen under conditions of performance stress (feeder and finishing operations) and in association with low-roughage diets high in digestible protein, unsaturated fat, and carbohydrates. Diets of this nature are thought to predispose pigs to ulceration by inducing the oversecretion

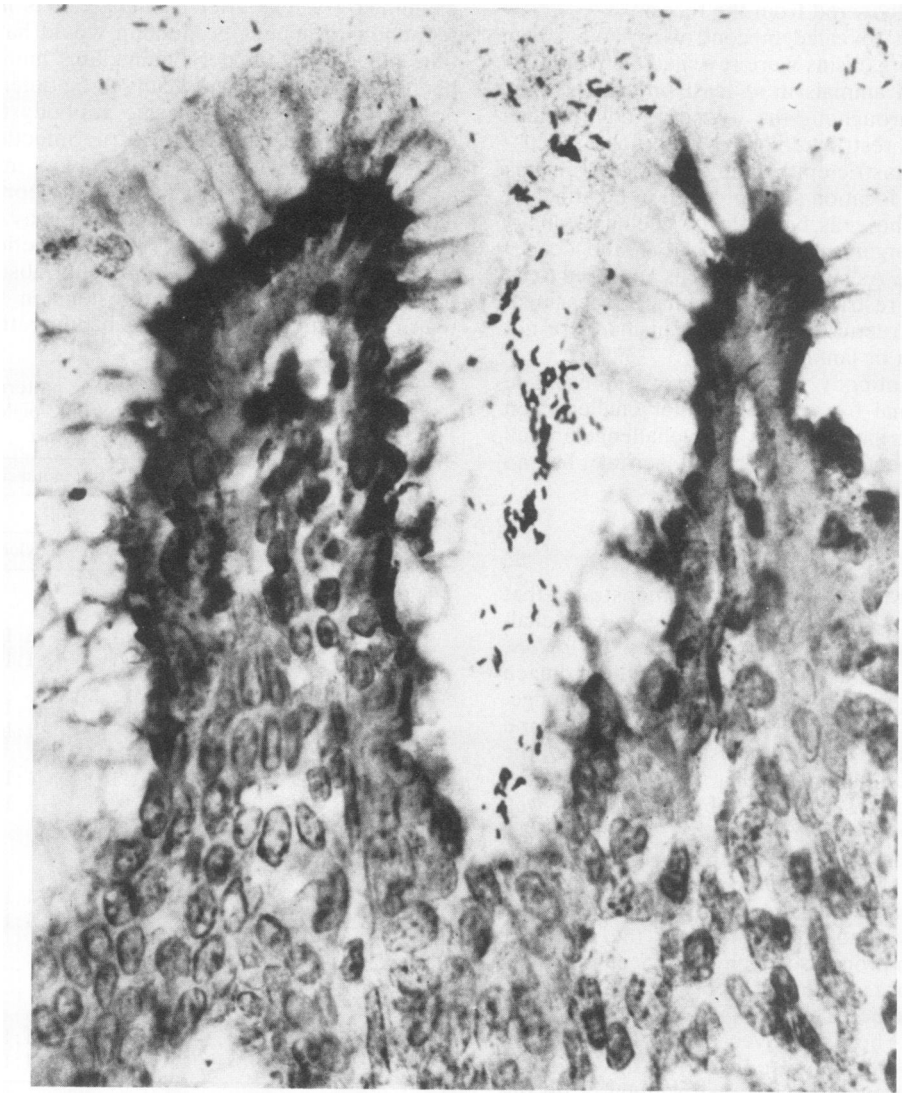

FIG. 4. *C. pylori* in the superficial mucus-secreting layer of the gastric epithelium in a piglet 6 days after challenge with *C. pylori*. WS silver stain.

TABLE 4. Antibody titers to *C. pylori* in sera of gnotobiotic control piglets and piglets challenged with *C. pylori*

Piglet no.	Sacrifice interval (wk)	Reciprocal titer[a] of *C. pylori*-specific immunoglobulin G
Litter 1		
86-1918 (Control)	1	5
86-1917 (Control)	3	5
86-1914	1	5
86-1915	1	5
86-1913	2	10
86-1916	2	80
86-1911	3	80
86-1912	3	80
Litter 2		
86-3229 (Control)	1	5
86-3227 (Control)	2	5
86-3228 (Control)	4	5
86-3221	1	5
86-3222	1	5
86-3223	2	5
86-3224	2	5
86-3225	4	80
86-3226	4	160

[a] Dilution factor of pig serum in the last well which produced a positive reaction (defined as twice the background).

of acid (via the release of histamine or serotonin) and delaying the emptying of the stomach (9). These factors are thought to disturb the natural pH gradient within the stomach, thereby permitting the reflux of acid cranially to effect a mucus-free and thus unprotected cardiac or esophageal epithelium. Support for this hypothesis is engendered by studies which have demonstrated that the oral administration of prostaglandin inhibitors (e.g., indomethacin) indirectly enhances the relative acidity within the organ by reducing the prostaglandin-mediated formation and release of bicarbonate by gastric mucus cells (9). Experimentally, ulceration can be produced in pigs by parenteral administration of histamine and reserpine (19, 20).

In humans, the pathogenesis of gastroduodenal ulcers is poorly understood (1). An inappropriate production of gastric acid is thought to be the main precipitating cause of this malady (6). Support for this concept comes largely from clinical observations which indicate that drugs which antagonize acid secretion, namely, cimetidine and ranitidine, promote the healing of ulcers. There can be no doubt that continued acid secretion promotes ulceration after initiation in the gastric mucosa. In an otherwise normal individual, it is difficult to envision a situation in which the overproduction of acid per se is a primary event. It is more likely that acid production is an important cofactor that perhaps functions to perpetuate and accentuate ulceration induced by other mechanisms. Gastritis induced by *C. pylori* may be the important and unrecognized initiator of this common human condition.

In summary, we have shown that neonatal gnotobiotic piglets are susceptible to infection by a human pathogen, *C. pylori*. The organism colonizes gastric mucosa and reproduces many features of the corresponding disease syndrome in humans. Further development and exploitation of this nonprimate animal model should permit the discovery of the pathogenic mechanisms of gastric disease associated with this agent and should ultimately provide insights into a more rational approach to the diagnosis and treatment of human gastric disease.

ACKNOWLEDGMENTS

We thank Nancy J. Austin for excellent technical assistance, Judith Dubena for the care and maintenance of piglets, J. Kowalski and Allan Lawrence for microbiology assistance and support, and Denise McCarthy and Ralph Bush for technical assistance with the enzyme-linked immunosorbent assay.

ADDENDUM

Since the original submission of our manuscript, Lambert and co-workers (13) published preliminary data for one litter of neonatal gnotobiotic piglets demonstrating gastric colonization and inflammation following pretreatment with ranitidine and oral challenge with 10^6 CFU of *C. pylori*. A similar challenge of conventional, colostrum-deprived piglets failed to result in gastric colonization or inflammation. Recent experiments in our laboratories demonstrated that challenge of conventional, colostrum-deprived piglets with 10^9 CFU of *C. pylori* also failed to result in gastric colonization or inflammation (manuscript in preparation).

LITERATURE CITED

1. **Booth, L., G. Holdstock, H. MacBride, P. Hawtin, J. R. Gibson, A. Ireland, J. Bamforth, C. E. DuBoulay, R. S. Lloyd, and A. D. Pearson.** 1986. Clinical importance of *Campylobacter pyloridis* and associated serum IgG and IgA antibody responses in patients undergoing upper gastrointestinal endoscopy. J. Clin. Pathol. **39**:215–219.
2. **Buck, G. F., W. K. Gourley, W. K. Lee, K. Subramanyam, J. M. Latinus, and A. R. DiNuzzo.** 1986. Relation of *Campylobacter pyloridis* to gastritis and peptic ulcer. J. Infect. Dis. **153**: 664–669.
3. **Cheli, R., and A. Giacosa.** 1986. Duodenal ulcer and chronic gastritis. Endoscopy **18**:125–126.
4. **Cheville, N. F.** 1980. Criteria for development of animal models of diseases of the gastrointestinal system. Am. J. Pathol. **101**:67–76.
5. **Gilman, R. H., R. Leon-Barua, J. Koch, A. Ramirez-Ramos, S. Recavarren, W. M. Spira, and C. B. Stephenson.** 1986. Rapid identification of pyloric *Campylobacter* in Peruvians with gastritis. Dig. Dis. Sci. **31**:1089–1094.
6. **Goodwin, C. S., J. A. Armstrong, and B. J. Marshall.** 1986. *Campylobacter pyloridis*, gastritis, and peptic ulceration. J. Clin. Pathol. **39**:353–365.
7. **Jones, D. M., J. Eldridge, A. J. Fox, P. Sethi, and P. J. Whorwell.** 1986. Antibody to the gastric campylobacter-like organism (*Campylobacter pyloridis*)—clinical correlations and distribution in the normal population. J. Med. Microbiol. **22**: 56–72.
8. **Jones, D. M., A. M. Lessels, and J. Eldridge.** 1984. *Campylobacter*-like organisms on the gastric mucosa: culture, histological, and serological studies. J. Clin. Pathol. **37**:1002–1006.
9. **Jubb, K. V. F., P. C. Kennedy, and N. Palmer.** 1985. Pathology of domestic animals, 3rd ed., vol. 2, p. 24–49. Academic Press, Inc., New York.
10. **Kaldor, J., W. Tee, P. McCarthy, J. Watson, and B. Dwyer.** 1985. Immune response to *Campylobacter pyloridis* in patients with peptic ulceration. Lancet **i**:921.
11. **Kasper, G., and N. Dickgieber.** 1984. Isolation of *Campylobacter*-like bacteria from gastric epithelium. Infection **12**:179–180.
12. **Kurihara-Bergstrom, L., M. Woodworth, S. Feisullin, and P. Beall.** 1986. Characterization of the Yucatan miniature pig skin and small intestine for pharmaceutical applications. Lab. Anim. Sci. **36**:398–399.
13. **Lambert, J. R., M. Borromea, H. Turner, M. G. Korman, and J. Hansky.** 1987. Colonization of gnotobiotic piglets with *Campylobacter pyloridis*. Gastroenterology **92**:1489.
14. **Langenberg, M. L., G. N. J. Tytgat, M. E. I. Schipper, P. J. G. M. Rietra, and H. C. Zaren.** 1984. *Campylobacter*-like organisms in the stomachs of patients and healthy individuals.

Lancet **i**:1348–1349.
15. **Marshall, B. J.** 1986. *Campylobacter pyloridis* and gastritis. J. Infect. Dis. **153**:650–657.
16. **Marshall, B. J., D. B. McGechie, G. J. Francis, and P. J. Utlet.** 1984. Pyloric *Campylobacter* serology. Lancet **ii**:281.
17. **Marshall, B. J., H. Royce, D. I. Annear, C. S. Goodwin, J. W. Pearman, J. R. Warren, and J. A. Armstrong.** 1984. Original isolation of *Campylobacter pyloridis* from human gastric mucosa. Microbios **25**:83–88.
18. **McDowell, E. M., and B. F. Trump.** 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. **100**:405–414.
19. **Muggenburg, B. A., T. Kowalczyk, W. G. Hoekstra, and R. H. Grummer.** 1966. Experimental production of gastric ulcers by reserpine. Am. J. Vet. Res. **27**:1663–1669.
20. **Muggenburg, B. A., T. Kowalczyk, N. A. Reese, W. G. Hoekstra, and R. H. Grummer.** 1966. Experimental production of gastric ulcers in swine by histamine in mineral oil-beeswax. Am. J. Vet. Res. **27**:292–299.
21. **Price, A. B., J. Leir, J. M. Dolby, P. L. Dunscombe, A. Smith, J. Clark, and M. L. Stephenson.** 1985. *Campylobacter pyloridis* in peptic ulcer disease: microbiology, pathology and scanning electron microscopy. Gut **26**:1183–1188.
22. **Rollason, T. P., J. Stone, and J. M. Rhodes.** 1984. Spiral organisms in endoscopic biopsies of the human stomach. J. Clin. Pathol. **37**:23–26.
23. **Tricottet, V., P. Bruneval, O. Vire, and J. P. Camilleri.** 1986. *Campylobacter*-like organisms and surface epithelium abnormalities in active chronic gastritis in humans: an ultrastructural study. Ultrastruct. Pathol. **10**:113–122.
24. **Tytgat, G. N. J., M. L. Langenberg, E. Rauws, and P. J. G. M. Rietra.** 1985. *Campylobacter*-like organisms (CLO) in the human stomach. Gastroenterology **88**:1620–1628.
25. **Waxler, G. L., D. A. Schmidt, and C. K. Whitehair.** 1966. Technique for rearing gnotobiotic pigs. Am. J. Vet. Res. **27**:300–307.