Isolation and preliminary characterization of a novel *Helicobacter* species from swine

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Objective—To determine whether a *Helicobacter* sp similar to *Helicobacter pylori* in the stomachs of humans could be isolated from the stomachs of pigs.

Animals—4 young conventionally reared and 21 gnotobiotic pigs.

Procedure—Gastric mucosal homogenates (10% wt/vol) from 4 young conventionally reared pigs were cultured on Skirrow medium under microaerophilic conditions to assess the presence of *Helicobacter* spp. Colonies with morphologic features compatible with *Helicobacter* organisms were selected, tested for urease activity, and subpassaged on Skirrow medium. Isolates were examined via SDS-PAGE electrophoresis and reciprocal western blot analyses involving convalescent sera from monoinfected gnotobiotic pigs.

Results—Urease- and catalase-positive, gram-negative, microaerophilic, small, curved rod bacteria were isolated from the gastric mucosa of young healthy pigs. The first isolate (2662) was structurally and immunologically closely related to *H pylori* isolated from humans. The second isolate (1268) displayed an SDS-PAGE profile dissimilar to that of *H pylori* and isolate 2662, yet it shared limited immunologic cross-reactivity with these microbes.

Conclusions and Clinical Relevance—Findings of this study indicate that development of gastric mucosal ulcers and ulceration of the nonglandular pars esophagea in pigs may be associated with gastric colonization by swine-origin *Helicobacter* spp, which are similar to *H pylori* isolated from humans. (*Am J Vet Res* 2005;66:938–944)

Helicobacter pylori is a gram-negative, motile, microaerophilic, gastric bacterial pathogen that is now accepted as the cause of human type B gastritis,[1,2] gastric and duodenal mucosal ulceration,[1-6] and gastric carcinoma.[1,2,7-10] Koch's postulates have been fulfilled in humans[11,12] and in models of these gastrointestinal tract diseases in swine.[13,14] Results of studies to investigate the epidemiology[1,7,15] and antimicrobial treatment[16,17] of *H pylori* infection and the pathologic changes[18-20] associated with such infection have provided overwhelming evidence of causality. The case for *H pylori* as the primary cause of nonsteroidal anti-inflammatory–independent ulceration of the gastric and duodenal mucosae in humans is strong because failure to eradicate organisms from these sites with antimicrobial treatments is associated with a high rate of ulcer recurrence[3-6] and long-term resolution of ulcerative disease follows eradication of *H pylori*.[16,17] Similarly, atrophic gastritis (considered to represent the last stage in the progression of long-term colonization of the stomach with *H pylori*) and interactions with other environmental factors[1,4,6] are now recognized risk factors for the development of gastric carcinoma.[1,2,7-10] Finally, low-grade B-cell gastric lymphoma is also associated with *H pylori* infection.[21-23] Since the first reports of *H pylori* infection and its association with disease in humans, related *Helicobacter* organisms have been detected in other species such as cats and dogs,[24-28] cheetahs,[29,30] and swine.[31-33]

Gastric ulceration is an important disease entity in swine.[34,35] Ulcerative lesions develop in both the glandular and nonglandular gastric mucosa of pigs; the prevalence of such lesions ranges from 5% to 100%. Death losses attributable to ulcerative disease may exceed 3%; economic losses resulting from subclinical hemorrhage and associated syndromes of anemia, anorexia, and weight loss are not known but are likely substantial.[34] Unlike humans or other carnivores, the corpus of the stomach of pigs contains an extension of the esophageal mucosa that is termed the pars esophagea. Ulceration of the nonglandular esophageal region in swine is pathologically similar to reflux gastritis and Barrett's esophagus in humans.[36,37] This region of the stomach of pigs is lined by stratified squamous epithelium; it is devoid of mucus-secreting cells and has no extracellular mucus layer nor a local bicarbonate production mechanism thought necessary to neutralize gastric acidity.[34,36] Gastric acid–mediated ulceration of the nonglandular esophageal mucosa is believed to be potentiated by the physical and nutrient content of the diet.[34-39]

In recent years, investigators have associated gastric colonization by *Helicobacter heilmannii* (formerly *Gastrospirillum suis*) with ulceration of the nonglandular esophageal region in pigs.[31-33,40-45] In contrast to other *Helicobacter* spp, this agent is large (10 to 12 µm) with a distinct spiral appearance[33]; generally, this organism cannot be cultured on artificial media.[40,43] Morphologically similar bacteria have been isolated from dogs,[25-27] cats,[28] and humans.[46] Although *H heilmannii* may be found anywhere in the glandular por-

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tion of the stomach of pigs, these bacteria have a preferential affinity for the fundus and acid-secreting parietal cells.[31,45] The organism (*H heilmannii* type 1) is an occasional human pathogen[46-49] but a common spiral *Helicobacter* sp in the stomachs of slaughterhouse swine.[32,41,44] Histologic inflammatory lesions associated with this agent are unremarkable and consist of lymphocytic and plasmacytic infiltrates and are typically most prominent in the antral region of the stomach.[44,45] *Helicobacter heilmannii* is infective for mice and has been transferred from mice to gnotobiotic pigs via a method that takes advantage of this bacterium's affinity for gastric parietal cells isolated from infected mice.[50] Yet, experimental challenge experiments performed by our group in gnotobiotic pigs[45] have failed to reveal the ulcerogenic potential of *H heilmannii* for swine.

The interest in the use of pigs as a model for human gastric bacterial disease is strong.[51-55] The ease with which gnotobiotic pigs can be colonized with many different human isolates of *H pylori*,[52] coupled with the fact that the porcine gastric mucosa expresses Lewis antigenic arrays similar to that of humans,[56,57] suggests that pigs possess a gastric niche that is highly compatible for colonization by *Helicobacter* organisms. A histologic survey performed by one of the authors involving stomachs collected from pigs after slaughter revealed the presence of *H pylori*-like bacteria attached to the mucosa of the cardiac and antral portions of the stomach; some of these putative *Helicobacter* organisms reacted immunohistochemically with *H pylori*-specific antisera. These data prompted us to test the hypothesis that swine raised in a conventional environment may harbor porcine gastric *Helicobacter* organisms that are more closely related to *H pylori* than to *H heilmannii*. The purpose of the study reported here was to determine whether a *Helicobacter* sp similar to *H pylori* in the stomachs of humans could be isolated from the stomachs of young conventionally reared pigs.

Materials and Methods

Swine—Four 6- to 7-week-old conventionally reared (ie, nongnotobiotic) pigs were purchased from a swine producer and transported to the laboratory. Food was withheld from the pigs for 12 hours; water was provided ad libitum. Pigs were sedated via IM administration of ketamine HCl (30 mg/kg); to obtain sera, blood samples were collected via jugular venipuncture. The pigs were then euthanatized by use of an IV injection of sodium pentobarbital (100 mg/kg). Polyclonal monospecific convalescent antisera against porcine gastric *Helicobacter* organisms were available from 36 gnotobiotic pigs[52] housed together by inoculation group. Thirteen, five, and nine 3-day-old gnotobiotic piglets were orally inoculated (**postinoculation day [PID]** 0) with 2 mL of broth cultures containing 10^8 viable active–growth-phase organisms of each of the isolates obtained in this study and *H pylori* strain 26695, respectively. Because *H heilmannii* cannot be cultured on conventional culture media, monospecific convalescent antisera against *H heilmannii* were collected from 6 gnotobiotic pigs inoculated at 3 days of age (PID 0) with gnotobiotic murine gastric homogenates containing the agent.[45] For immunoassays, sera were collected on PID 35. All animals used in this study were housed according to The Ohio State University Institutional Laboratory Animal Care and Use Committee guidelines and approvals; the university site is regularly inspected via visits (announced and unannounced) from USDA inspectors.

Sample collections from young conventionally reared pigs—After euthanasia, the stomach of each of the young pigs was isolated by ligation at the esophagus and proximal portion of the duodenum, removed, and opened along the greater curvature starting at the pylorus and following the gastrosplenic ligament attachment cranially to the esophagus. Any remaining food contents were removed, and the mucosal surface was gently rinsed with sterile saline (0.9% NaCl) solution. Each opened stomach was placed on a dissection tray; multiple mucosal strips (10/pig) measuring 5 × 15 mm were collected from the mucosae of the fundus and from the cardiac and antral portions of the stomach and placed in sterile Petri dishes for bacterial culture and isolation.

Histologic evaluation of tissue samples—Once the sample collections for microbiologic assessment were completed, the remaining stomach was preserved via fixation in 10% (vol/vol) formalin in PBS solution. After 24 hours of fixation, multiple samples from the cardiac portion of the stomach as well as from the fundus and antrum were collected from each pig, placed into histology cassettes, dehydrated, and embedded in paraffin by routine methods. Replicates of sections (5 µm) were stained with H&E and Warthin-Starry stains and examined for lesions and the presence of microbes by use of conventional histologic methods.[52]

Microbiologic assessments—Each mucosal piece was streaked onto a half portion of 2 plates containing Skirrow medium: 1 plate was precoated with amphotericin B (0.5 mg/plate), and the other was not precoated with amphotericin B. By use of an inoculating loop, substreaks from the initial streak were made onto the other half of each plate. In addition, mucosal samples from each pig were pooled, weighed, diluted to 10% (wt/vol) with fresh *Brucella* broth, and homogenized in sterile 10-mL-volume Ten Brock tissue grinders. Ten-fold dilutions of these homogenates were plated onto plates containing fresh Skirrow medium and incubated in a low-oxygen tension environment at 37°C for 4 days (**passage [p]**0). For each p1, colonies that were not obviously contaminated with other organisms and also had the typical colony morphology of *Helicobacter* spp were swabbed onto fresh trypticase soy agar plates containing 5% (vol/vol) sheep blood and incubated in a low oxygen tension environment at 37°C for 2 to 3 days (p2). After p2, p3, and p4, plates were again inspected; suspect colonies were selected and streaked onto trypticase soy agar containing 5% (vol/vol) sheep blood as before. After culture, each swab was evaluated for urease activity by placing it into urease-indicator solution containing 2.0% (wt/vol) urea and 0.5% phenol red in PBS solution (pH, 6.3 to 6.5); at p3, 1 swab was positive for urease. Of the 11 gastric isolates recovered from p4, 2 were positive for urease, 4 were equivocal for urease activity, and the remaining 5 were negative for urease. Two isolates were selected for further study and growth in *Brucella* broth containing 10% (vol/vol) heat-inactivated (56°C for 1 hour) fetal bovine serum. Cytospin preparations from each isolate were prepared, fixed in formalin, and stained with polyclonal rabbit antisera against *H pylori*.[a] During the isolation process, *H pylori* was neither passaged nor propagated in the laboratory and in vivo challenge experiments involving *H pylori* were not performed, thereby avoiding inadvertent contamination of specimens with laboratory-origin *H pylori*.

ELISA, SDS-PAGE electrophoresis, and western blot analyses—Microtiter plate ELISAs were constructed with sonicates of *H pylori* and urease-positive isolates (100 to 500 µg of bacterial sonicates in basic bicarbonate coating buffer [pH, 9.0]) as antigen, essentially as described previously.[52,53] Individual and pooled convalescent sera from gnotobiotic pigs that were inoculated orally with each gastric isolate obtained

in this study and *H pylori* were used to derive optimal test conditions for development of ELISA immunoassays. Goat anti-porcine IgM and IgG isotype-specific secondary reagents, coupled to horseradish peroxidase, were used. Microtiter wells were developed with 3,3',5,5'tetramethylbenzidine microwell substrate, and **optical densities (ODs)** were read at 450 nm. Each serum sample was tested in triplicate; the OD values were averaged to derive the mean titer of each sample.

For SDS-PAGE analyses,[54] both porcine isolates obtained in this study were propagated in *Brucella* broth. For comparison, *H pylori* strain 26695 was similarly prepared. One microliter of each sample (2.4 to 3.0 μg) was diluted 1:15 in distilled water and then mixed with an equal volume of loading buffer (Tris-Cl [50mM] and di-thio-threitol [100mM]) containing 2.0% SDS, 0.1% bromophenol blue, and 10% glycerol; this mixture was boiled for 3 minutes. Samples were then loaded onto PAGE gels and electrophoresed (60 to 75 minutes at 100 V) in Tris-glycine electrophoresis buffer (25mM Tris, 250mM glycine, and 0.1% SDS). Gels were stained with Coomassie blue, destained, rehydrated, and photographed.

For western blot analyses,[54] replicate samples of *H pylori* and the porcine isolates obtained in this study (1.1 to 4.5 mg/mL in 15% glycerol) were separated on PAGE gels as described and then transferred to nitrocellulose filters with a semidry transfer apparatus. Blots were blocked by incubation in PBS solution containing 10% (vol/vol) nonfat dry milk and 0.05% Tween 20 at 4°C for 12 hours. The nitrocellulose strips were then washed in PBS solution and incubated with a 1:15 dilution of pooled convalescent (PID 35) sera from 3 to 5 pigs for 2 hours at 22°C or with sera from gnotobiotic pigs inoculated with *H pylori* 26695, the first isolate obtained in this study, the second isolate obtained in this study, or *H heilmannii*. Blotted strips were washed 3 times (5 minutes/wash) and then incubated with goat anti-porcine IgG or IgM (diluted 1:250 in PBS-Tween 20 solution) for 1 hour at 22°C. This was followed by similar incubation with horse anti-goat IgG coupled to horseradish peroxidase (diluted 1:2,000 in PBS-Tween 20 solution). Blots were developed with the addition of 3,3',5,5'tetramethylbenzidine; the membranes were washed in distilled water to stop the reactions. Blotted membranes were digitally photographed, and molecular weight standards and other identifying features were superimposed on the digital images.

Results

Conventionally reared pigs—In all samples of the gastric mucosa that were obtained for bacteriologic culture from the conventionally reared pigs, lesions compatible with *Helicobacter* infection in this species[51,52] were detected histologically. These lesions consisted of multifocal to diffuse lymphocytic inflammatory cell infiltrates and lymphoid follicle development. In addition, occasional neutrophilic infiltrates and exudation into the glandular lumens were detected; 1 of the 4 pigs had small active mucosal ulcers in the antrum of the stomach. In the Warthin-Starry–stained sections of mucosae from all of the pigs, no *H heilmannii*-like spiral bacilli were

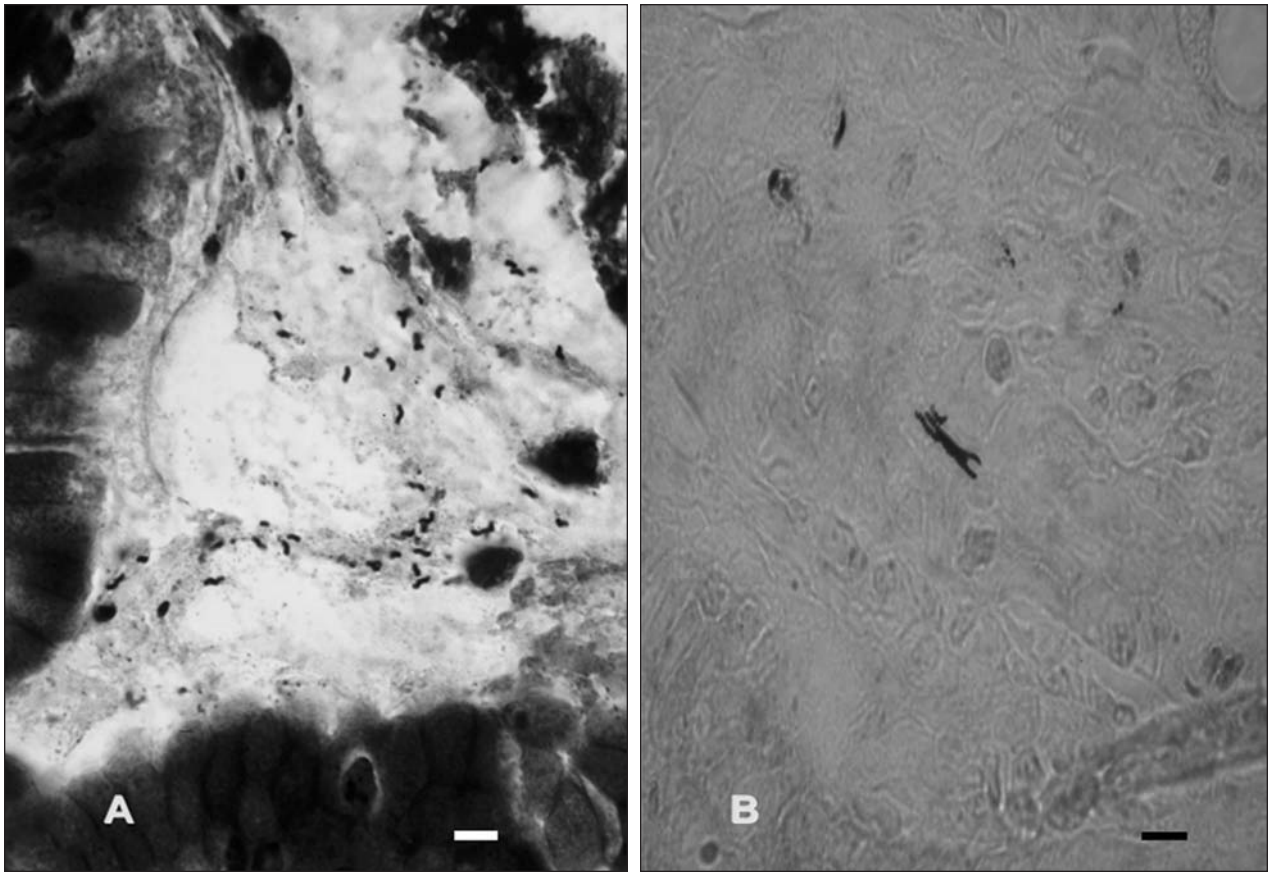

Figure 1—Photomicrographs of the appearance of *Helicobacter* spp in sections of gastric tissue obtained postmortem from young pigs. A—Section of gastric mucosa obtained from a young conventionally reared pig on day 35 after oral inoculation with porcine *Helicobacter* isolate 2662. B—Section of gastric mucosa obtained from a young gnotobiotic pig on day 35 after oral inoculation with *Helicobacter heilmannii* type 1. Warthin-Starry silver stain; bar = 10 μm.

detected. However, all sections contained small, extracellular, curved rod bacteria and occasional gull-wing–shaped rod bacteria in close apposition to the gastric epithelia and in the gastric mucus; these bacteria were compatible with the morphologic appearance of *H pylori* in humans and swine (**Figure 1**).[51] Results of the ELISA indicated that the sera of all pigs from which tissue samples were collected for bacteriologic culture contained moderate (0.65 to 0.79) to high (1.13 to 1.24) OD-unit titers of *H pylori*-reactive IgG antibodies.

Microbiologic assessments—Serial passage of urease-positive bacterial colonies (grown in culture of samples of gastric mucosa from 2 pigs) that had gross morphologic features compatible with those of *Helicobacter* organisms (ie, small, translucent pinpoint colonies on both Skirrow and trypticase soy agar-blood agar plates) yielded 2 organisms with different urease activity. The first putative *Helicobacter* isolate (designated isolate 2662) was characterized by a rapid hydrolysis of the urea reagent (ie, strong urease activity); robust catalase activity; and morphologic features that resembled short, gram-negative, curved, motile rod bacteria. On agar plates, this isolate grew as small translucent colonies, which are typical of *Helicobacter* spp. The second putative *Helicobacter* isolate (designated isolate 1268) was morphologically indistinguishable from the first (ie, gram-negative, short, curved rod bacteria and gull-wing–shaped rod bacteria); however, the second isolate was distinguished from the first by slow hydrolysis of the urea reagent (ie, slow urease activity) and a weak but definite catalase reaction. Cytospin preparations from each isolate were prepared, fixed in formalin, and stained with polyclonal rabbit antisera against *H pylori*; isolate 2662 reacted strongly, whereas isolate 1268 reacted positively (but weakly).

Results of SDS-PAGE protein analyses—Isolates 1268 and 2662, along with *H pylori* 26695, were subjected to SDS-PAGE gel analyses. Separated proteins were stained with Coomassie blue, and the resultant protein profiles were photographed (**Figure 2**). When compared with the positive-control profile for *H pylori*, isolate 2662 had a protein profile quite similar to that of *H pylori*. Additional gels that were stained with a silver stain revealed that isolate 2662 lacked 1 protein of approximately 30 to 35 kd and several high–molecular-weight (> 90 kd) proteins, compared with those identified in *H pylori*. In contrast, isolate 1268 was substantially different from both *H pylori* and isolate 2662; compared with findings in the latter 2 organisms, prominent bands at 40 to 50 kd and a strong 60-kd band were not present in isolate 1268. In addition, isolate 1268 contained a prominent band of approximately 70 kd, which was either not present or faintly represented in *H pylori* 26695 and isolate 2662.

Results of ELISAs—In microtiter ELISAs, each isolate was tested against both individual and pooled (3 to 4 pigs) convalescent serum samples from *H pylori*-, isolate 2662-, isolate 1268-, and *H heilmannii*-inoculated gnotobiotic pigs. Convalescent sera from *H pylori*-inoculated and isolate 2662-inoculated pigs reacted equally well with lysates prepared from *H pylori* and isolate 2662. These same antigen panels yielded negative results when tested with isolate 1268 and *H heilmannii* convalescent sera. Sera from uninfected control pigs yielded negative results via ELISAs. To exclude a low-level infection (and hence delayed serologic response) in pigs inoculated with isolate 1268 as an explanation for the lack of binding to *H pylori* and isolate 2662 antigens, sera were also evaluated for antibody activity of the IgM isotype; all sera yielded negative results via ELISAs (data not shown). Finally, ELISAs performed with 1268 sonicate yielded positive results for sera from all 5 isolate 1268-inoculated pigs and negative results for convalescent sera collected from *H pylori*-, isolate 2662-, and *H heilmannii*-inoculated and uninfected control pigs. As an additional control for ELISA specificity, sera from gnotobiotic pigs that had been inoculated with *H heilmannii*[45] were also evaluated in the ELISA; all of these sera yielded negative results via ELISA, whereas all sera collected from gnotobiotic swine inoculated with *H pylori* had high titers of antibodies against *H pylori* within 2 to 3 weeks after experimental infection (data not shown).

Results of western blot analyses—Pooled convalescent sera from *H pylori*-, isolate 2662-, isolate 1268-, or *H heilmannii*-inoculated and uninfected control pigs were blotted against SDS-PAGE–separated proteins from *H pylori*, isolate 2662, and isolate 1268 (**Figures 3 and 4**). Sera from uninfected control pigs did not react with any separated proteins (data not shown). Pooled antisera from *H pylori*-inoculated pigs

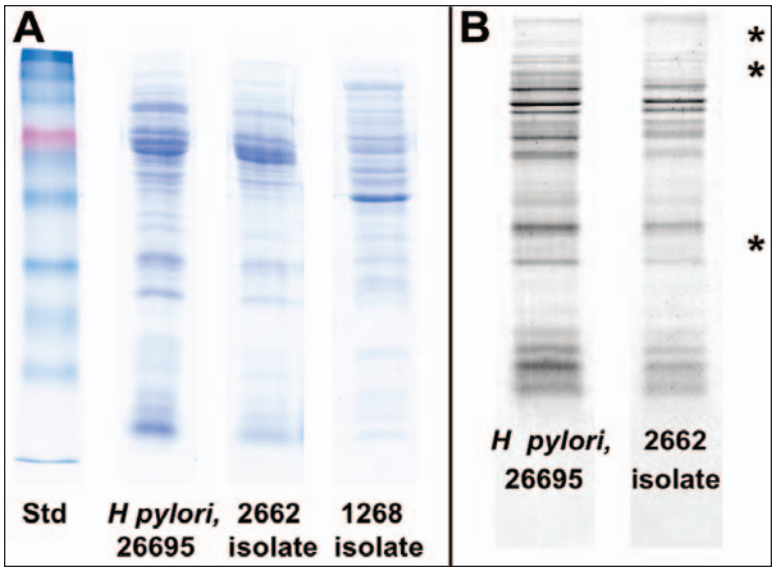

Figure 2—Representative SDS-PAGE profiles of *Helicobacter pylori* strain 26695 (positive control for the *Helicobacter* protein profile) and 2 *Helicobacter* isolates (2662 and 1268) obtained from young conventionally reared pigs. A—Gel stained with Coomassie blue. B—Gel stained with silver stains. The lane to the left of panel A contains molecular weight standards (Std). Asterisks identify proteins that are apparently present in *H pylori* but not in isolate 2662.

reacted with 6 or 7 proteins common to both *H pylori* and isolate 2662, but not to proteins of isolate 1268. Sera from *H heilmannii*-inoculated pigs bound one 70-kd protein common to *H pylori* and to isolates 1268 and 2662 and bound a second 30- to 35-kd protein common to *H pylori* and isolate 2662, but not to isolate 1268 (Figure 3).

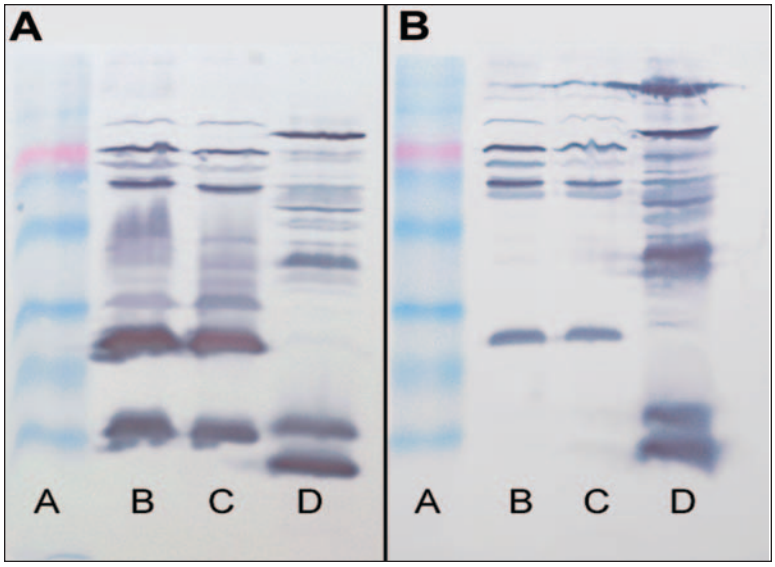

Figure 3—Western blot analyses of the antibody response profiles of convalescent sera obtained from isolate 2662-inoculated (A) and isolate 1268-inoculated (B) young gnotobiotic pigs when blotted against *H pylori* strain 26695 (lanes B), isolate 2662 (lanes C), and isolate 1268 (lanes D). For both blots, molecular weight standards are presented on the same blots (lanes A). Notice that pooled sera from isolate 2662-inoculated pigs reacts with at least 10 protein bands that are common to *H pylori* and isolate 2662 and with 4 or 5 proteins that are common to all 3 organisms. In addition, at least 5 proteins unique to isolate 1268 are identified (panel A, lane D). Pooled convalescent sera from isolate 1268-inoculated pigs reacts with approximately 30 protein bands expressed by isolate 1268 and 7 protein bands contained in isolate 2662 (panel B, lanes B and C), 5 of which appear to be common to isolate 1268 (panel B, lane D). Blots were stained with goat anti-porcine IgG (1:250) followed by equine anti-goat IgG (1:2,000) and developed with 3,3',5,5' tetramethylbenzidine.

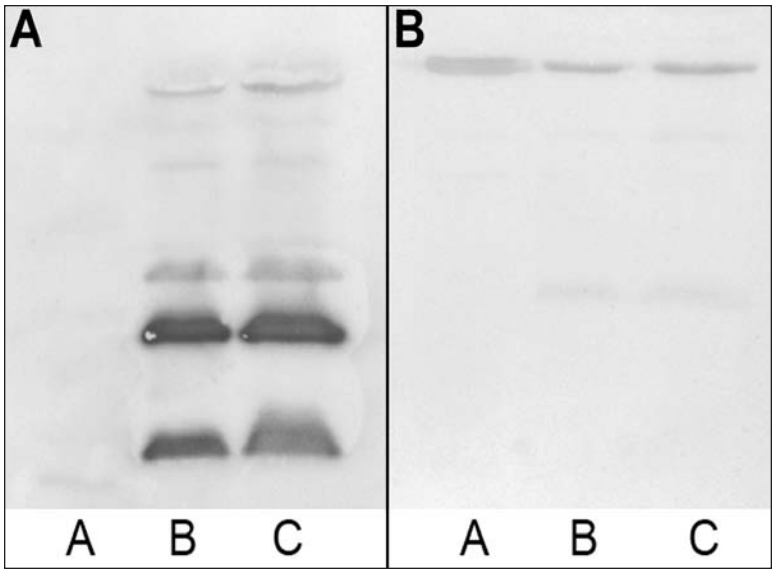

Figure 4—Western blot analyses of the antibody response profiles of convalescent sera obtained from *H pylori*-inoculated (A) and *H heilmannii*-inoculated (B) young gnotobiotic pigs when blotted against isolate 1278 (lanes A), isolate 2662 (lanes B), and *H pylori* strain 26695 (lanes C). Notice that pooled sera from *H pylori*-inoculated pigs reacts with at least 7 protein bands common to *H pylori* and isolate 2662 and with no proteins from isolate 1268. Pooled sera from *H heilmannii*-inoculated pigs reacts with 1 protein band that is common to *H pylori*, isolate 1268, and isolate 2662 and 1 additional band that is common to *H pylori* and isolate 2662. Blots were stained with goat anti-porcine IgG (1:250) followed by equine anti-goat IgG (1:2,000) and developed with 3,3',5,5' tetramethylbenzidine.

In contrast, convalescent sera from isolate 2662-inoculated pigs reacted with 9 protein bands that were common to both *H pylori* and isolate 2662 as well as with 9 proteins in isolate 2662 that were distinctly different from the those bands recognized by immune sera from *H pylori*-inoculated pigs. Pooled convalescent sera from pigs inoculated with isolate 1268 reacted with at least 7 proteins that were common to *H pylori* and isolate 2662 and with approximately 18 immunogenic protein bands contained within isolate 1268. Of these 18 bands, 6 appeared to be common to both *H pylori* and isolate 2662 (Figure 4).

Discussion

Gastric ulcer disease is a common disease entity in pigs of slaughter age.[34,35] The ulcerative lesions in the pars esophagea can result in less than optimal performance, intragastric hemorrhage, and death due to exsanguination into the gastric lumen. Ulceration of the gastric glandular mucosa, primarily in the gastric antrum and lesser curvature, also develops with some frequency; in our experience, fresh and healed mucosal ulcers are common findings in pigs that have been slaughtered and are occasionally detected in gnotobiotic pigs inoculated with *H pylori*.[55] Development of these ulcerative lesions is attributed to high-carbohydrate and finely ground rations,[34] the effects of gastric acid reflux,[38,39] or the action of acidic metabolites of microbial carbohydrate metabolism[45] on the unprotected pars esophagea. In recent years, ulceration of the nonglandular esophageal region and mucosae of the stomach of pigs has been attributed in part to gastric colonization by *Helicobacter*-like species, notably the spiral bacterium named *G suis* or *H heilmannii*.[31,32,40-44] On the basis of detection of ureA and cagA genes in gastric biopsy specimens[43] and results of 16S ribosomal RNA typing,[40] investigators have concluded that these microbes are > 95% homologous to *H heilmannii* type 1 detected in humans. Detailed analysis of *H heilmannii* is hampered by the inability of many investigators, including ourselves, to culture this agent on artificial media. These organisms are common gastric inhabitants of swine,[31,32,41,44] and the stomachs of as many as 50% of pigs without ulcers are colonized by these bacteria. The gastric niche preferred by *H heilmannii* is predominantly the gastric fundus and pits, often in

close association with gastric parietal cells. In fact, a proportion of these bacteria may actually be found within the canicular system of parietal cells. It is this last feature that has permitted our group to recover pure cultures of *H heilmannii* from isolated infected murine parietal cells for pathogenesis studies[50,52] in gnotobiotic pigs. Our attempts to implicate *H heilmannii* as a porcine gastric ulcerogen through experiments involving inoculation of this agent into gnotobiotic pigs with or without fermentable carbohydrate nutritional supplementation have failed.[45] Thus, although there may be an association between *H heilmannii* gastric colonization and ulceration of the nonglandular esophageal region, the fact that the agent is not ordinarily found in direct association with gastric lesions and that many *H heilmannii*-infected pigs are ulcer-free[32,42,44] suggests that this association is not a direct cause-and-effect relationship.

In the study of this report, a *Helicobacter* sp (especially isolate 2662) was recovered from healthy young pigs; the organism is closely related to *H pylori* isolated from humans but morphologically distinct from *H heilmannii*. This isolate has an SDS-PAGE profile similar to that of *H pylori*; it cross-reacts with *H pylori* in ELISAs and with both *H pylori* and *H heilmannii* in western blot immunoassays and intradermal cutaneous delayed-type hypersensitivity assays (data not shown). Moreover, as we reported elsewhere,[58] isolate 2662 induces prominent gastric inflammation and is ulcerogenic after oral inoculation into gnotobiotic pigs. We have also determined in other experiments that isolate 2662 is both urease- and catalase-positive and possesses the cagA gene cluster, a known virulence marker for human *H pylori*. The second isolate (1268) identified in the present study was also urease-positive and morphologically similar to *H pylori*; it appears to be distantly related to both isolate 2662 and *H pylori* on the basis of a dissimilar SDS-PAGE profile and minimal serologic cross-reactivity to *H pylori* when evaluated via ELISAs and western blot analyses, respectively. Gnotobiotic pigs that were inoculated with isolate 1268 developed minimal inflammatory lesions in their stomachs.

Although isolate 2662 contains the major proteins typical of *H pylori* isolated from humans, this organism induces a different antibody response pattern in inoculated gnotobiotic pigs, compared with the antibody response pattern induced by *H pylori* in gnotobiotic pigs. Whereas pooled convalescent sera from *H pylori*-inoculated pigs did not contain antibodies against isolate 1268 when evaluated via western blot analyses, pooled convalescent sera from isolate 2662-inoculated pigs contained antibodies that had specificity for several different proteins contained in isolate 1268 (with which sera from *H pylori*-inoculated pigs did not react). These data suggest that there are important immunologic differences between *H pylori* and isolate 2662 and confirm that porcine isolate 2662 is antigenically distinct from *H pylori*.

Recovery and culture of *Helicobacter* spp from the porcine gastric microenvironment are technically challenging. Other investigators have attempted this but have not been successful.[40,44] In this regard, it is perhaps notable that all pigs that were used for recovery of porcine *Helicobacter* organisms in the present study were seropositive for *H pylori* antigens (as determined via ELISAs), yet organisms were recovered from only 2 of 4 pigs. Importantly, sera from *H heilmannii*-inoculated gnotobiotic pigs did not yield positive ELISA results when evaluated against *H pylori* or isolate 2662 bacterial antigens. These findings indicate that the antibodies detected via ELISA were not identified because of serologic cross-reactions to antigens common to isolate 2662 and *H heilmannii*. Preliminary attempts in this laboratory to recover *Helicobacter* organisms from stomachs collected from swine in slaughterhouses were unsuccessful, in part because of the heavy overgrowth of contaminant microbes on the *Helicobacter*-selective medium. Also, unlike the pigs included in our study, food had not been withheld from those slaughterhouse swine for 24 hours prior to slaughter. Moreover, the use of younger pigs (5 to 6 weeks of age) in the present study may have contributed to our success in these recent experiments. Whatever the reason, 2 isolates were obtained from the pigs used in our study; moreover, it is evident that one of those isolates (2662) is very similar to *H pylori* isolated from humans. Thus, it is not surprising that this isolate is pathogenic in gnotobiotic pigs. Results of a preliminary serologic study conducted by our group have indicated that seroconversion against *Helicobacter* antigens (both *H pylori* and isolate 2662) occurs in conventionally reared pigs at 4 to 6 weeks of age and that 70% to 80% of pigs that are old enough to be slaughtered are seropositive for *Helicobacter* antigens. Although more extensive epidemiologic studies must be performed with this isolate in conventionally reared swine before firm conclusions can be reached, these data strongly suggest that pigs may commonly contain *Helicobacter* spp in their stomachs, these organisms are closely related to *H pylori* isolated from humans but distinct from *H heilmannii* type 1, and infection with these *H pylori*-like bacteria may be an important infectious component of gastric ulcerogenesis in pigs. Moreover, these results hold the promise that vaccination of swine against *H pylori*-like bacteria may be an as yet unexamined prophylactic measure to prevent the development of gastric ulcers in pigs, which is commonly encountered in commercial swine operations.

a. Rabbit anti-*H pylori* antibody, Novacastra Vision BioSystems, Norwell, Mass, and DakoCytomation Inc, Carpinteria, Calif.

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