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Newborn Piglet Model for Campylobacteriosis

FARAH K. BABAKHANI,† GREGORY A. BRADLEY, AND LYNN A. JOENS*
Department of Veterinary Sciences, University of Arizona, Tucson, Arizona 85721

An in vivo model system for human campylobacteriosis has been developed in which colostrum-deprived newborn piglets are orally challenged with an invasive strain of *Campylobacter jejuni*. Piglets developed clinical symptoms and histopathological lesions similar to those observed in humans infected with *C. jejuni*. Gross lesion examination at autopsy revealed the presence of edema, hyperemia, and mucus. Histopathologic examinations by light and transmission electron microscopy demonstrated damage to surface epithelial cells with the presence of intracellular bacteria, mainly in the large intestine. Similar lesions were not demonstrated in control piglets.

The recognition of *Campylobacter jejuni* as one of the leading causes of human enteritis throughout the world (23) was not possible until the advancement of proper culture techniques in the 1970s (4, 29). Two decades of laboratory research on *C. jejuni* suggests that the pathogenicity of the organism may be due to a variety of virulence factors such as enterotoxins, cytotoxins, and invasins. However, the biological significance of these virulence factors is unclear. The lack of progress in clarifying the role of toxins and invasive properties of *C. jejuni* during the disease process is partly due to the absence of a suitable animal model that would mimic human campylobacteriosis.

The standard animal model systems used with other enteric pathogens such as the Sereny test or ligated rabbit ileal loops have been found to be negative for *C. jejuni* (17, 23). Therefore, various other animals have been experimentally inoculated with *C. jejuni* in an attempt to reproduce the symptoms of *Campylobacter* enteritis (2, 5, 11, 12, 15, 16, 19, 24, 26–28, 30, 33). Some of these studies have investigated animal models that mimic complications seen in human *Campylobacter* infection, such as hepatitis or abortion (2, 19). Other studies have shown pathological changes to goblet cells and host death only after the host was treated with iron-dextran and magnesium or infected with *Cryptosporidium parvum* (8, 18, 32). With the exception of a few reports, the remainder of the studies have resulted either in asymptomatic transient colonization with bacterial shedding or mild symptoms with little associated histopathological intestinal damage.

Few investigators have been able to reproduce clinical symptoms of campylobacteriosis. These studies either have used hosts that are not similar to humans (6, 30) and are expensive to maintain (15, 27) or have produced inconsistent results. A notable study was performed with gnotobiotic beagle puppies by Prescott et al. (27). These investigators orally inoculated the animals with *C. jejuni* of human and canine origin. Although clinical manifestations of disease were seen, they were milder in the animals than in humans infected with *C. jejuni*. Human disease is accompanied by abdominal pain, fever, and the presence of blood and mucus in stools, whereas the puppies became bacteremic and developed only mild diarrhea. Histopathologically, the puppies demonstrated mild colitis consisting of neutrophil infiltration in the lamina propria, loss of goblet cells with hypertrophy of glands, and exfoliation of surface epithelium. A removable intestinal tie adult rabbit diarrhea model was used by Caldwell et al. (6) to establish infection with *C. jejuni*. In this model, temporary ligation of the rabbit ileum followed by inoculation of live bacteria resulted in disease similar to that observed in humans, albeit with a higher incidence of bacteremia and death. However, the surgical manipulations required by such a model make it a costly and highly technical procedure. In a separate study, Ruiz-Palacios et al. (28) produced clinical symptoms of *Campylobacter* infections in young chickens. However, attempts by other investigators have not produced similar results (5, 24, 27).

Despite experimentation with many varieties of animals, a suitable and inexpensive in vivo model system which could mimic human *Campylobacter* enteritis has not yet been found. Partial success has been achieved only in germfree young animals (15, 16, 26, 30) and, inconsistently, in chickens (28). A suitable model system requires a host that is similar to humans. This would allow the study of both bacterial virulence and host immune mechanisms. The cost associated with rearing and maintaining hosts for model systems is also a determinant factor in choosing a suitable model. The role of specific immunity in *Campylobacter* infection implies that a third factor, namely, the host defense system, must be taken into consideration when searching for a suitable model system (1, 2, 25).

The present study describes a practical animal model using colostrum-deprived newborn piglets to mimic human campylobacteriosis. A previous observation of *Campylobacter*-associated enteric disease in these animals (31) supports their use in investigating virulence factors associated with human campylobacteriosis. The lack of competing intestinal flora, the absence of maternal antibodies, and similarities of digestive systems make newborn piglets a preferred in vivo model for human campylobacteriosis.

MATERIALS AND METHODS

Inoculum. *C. jejuni* M129 from a patient with clinical signs of campylobacteriosis was kindly provided by Kenneth Ryan (University Medical Center, University of Arizona, Tucson). This isolate was kept frozen in liquid nitrogen in bovine blood. Previous observations by Konkel et al. indicated that a low-passage M129 strain (nine or fewer passages) was able to invade tissue culture cells at significantly high numbers (0.1%) (21, 22). Therefore, for experimental

* Corresponding author.
† Present address: Department of Pathology, Medical College of Virginia, Richmond, VA 23298.

TABLE 1. Clinical symptoms of neonatal pigs infected with bacteria

Pig no.	Clinical symptoms[a] on:																	
	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	D	B	M	D	B	M	D	B	M	D	B	M	D	B	M	D	B	M
C. jejuni infected																		
1	+	+	+	+	+		N	N	N									
2	+	+		−	−	−	+	+	+	+	+	−	−	−	−	N	N	N
3	+	+	−	+	+	+	+	+	−	N	N	N						
4	+	+	+	+	+	−	+	−	+	+	−	+	+	+	+	N	N	N
5	−	−	−	+	−	+	+	+	−	+	+	−	−	−	−	N	N	N
6	+	+	−	−	−	−	+	+	+	+	−	+	+	−	−	N	N	N
7	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	N	N	N
E. coli infected																		
8	−	−	−	−	−	−	N	N	N									
9	−	−	−	−	−	−	+	−	−	N	N	N						
10	−	−	−	+	+	−	+	+	−	+	+	−	+	+	−	N	N	N

[a] Clinical symptoms observed included diarrhea (D), blood in the stools (B), and mucus in the stools (M). +, presence; −, absence; N, piglets were necropsied.

assays, *C. jejuni* M129 passaged fewer than nine times was grown microaerophilically (N_2-CO_2-H_2, 80:10:10) at 37°C on Mueller-Hinton (MH) agar plates (eight plates) containing 4% citrated bovine blood. A noninvasive *Escherichia coli* strain, LE392 [F^- *hsdR57* (r^- m^+) *supE44 supF58 lacY1 galK2 galT22 metB1 trpR55* λ^-] (Promega, Madison, Wis.), was used as the control strain and grown aerobically on MH plates (two plates). Twenty-four-hour cultures of *C. jejuni* and *E. coli* were harvested and used to inoculate newborn piglets.

Piglets. Ten colostrum-deprived newborn piglets (K & K Pork, Chandler, Ariz.) were used in the present study. These piglets were obtained from the dams on three separate occasions. Each time, they were rinsed with Betadine and transported in sterile boxes to well-ventilated isolation units. Experimental and control piglets were kept separately in different isolation units. Each piglet was fed approximately 60 ml of Similac (Ross Laboratories, Colombus, Ohio) three times daily.

Animal inoculation. For experimental inoculation, 24-h cultures of *C. jejuni* and *E. coli* were harvested from plates with phosphate-buffered saline (PBS). The suspensions were centrifuged at 6,000 × *g* for 10 min at 4°C. The pellets were resuspended in Similac and adjusted to yield between 1×10^8 and 7×10^8 viable organisms per ml. Prior to challenge, newborn piglets were made to fast for approximately 8 h and then orally inoculated with approximately 40 ml of either *C. jejuni* M129 or *E. coli* LE392. The inoculum size was chosen on the basis of preliminary reports with a swine loop model (data not shown) and a previous report with primate models (15).

Observations. All of the piglets were observed daily for clinical signs (fecal consistency, presence of occult blood and mucus in stools, and general appearance) of disease, and the results were recorded. The occurrence of diarrhea was indicated by subjective evaluation of large amounts of loose fecal discharge present in the cages of each piglet. The presence of occult blood in feces was determined by using Hemoccult slides (SKD Inc., San Jose, Calif.). Rectal swabs were applied to Hemoccult slides as described in the manufacturer's instructions.

Isolation of bacteria. Fecal samples, prior to inoculation and following necropsy, were cultured on three different media: (i) Tergitol 7 agar (Difco, Detroit, Mich.), (ii) Tryp-

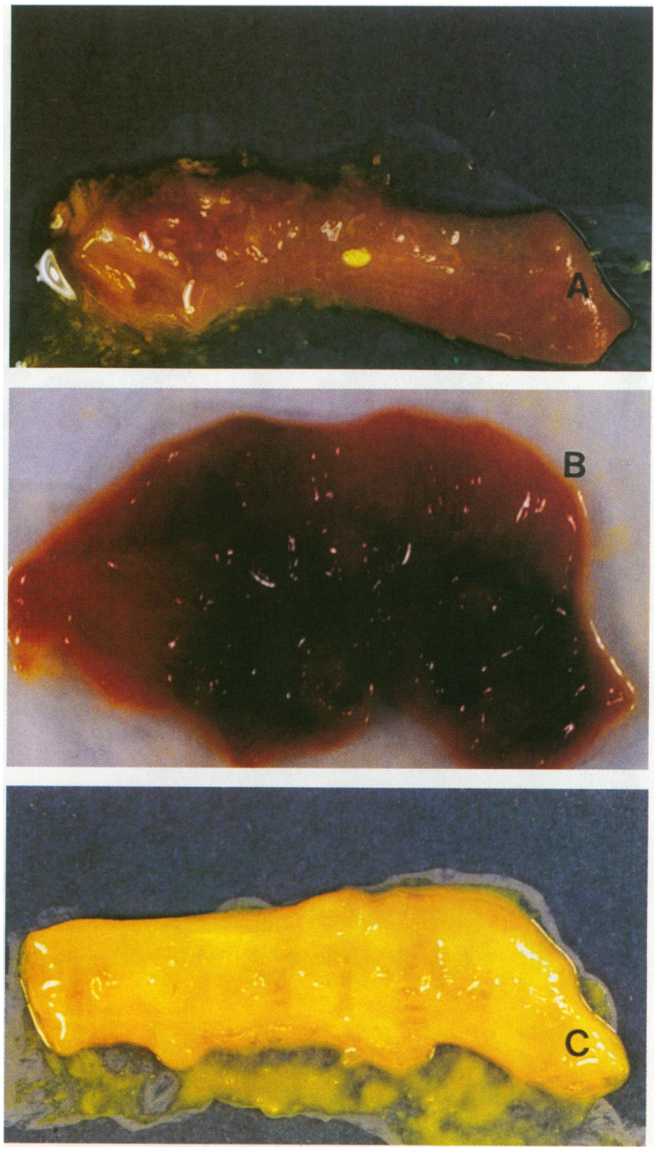

FIG. 1. Large intestine from neonatal pigs infected with *C. jejuni* at 3 (A) or 6 (B) days p.i. or with *E. coli* (C).

TABLE 2. Postnecropsy gross examination of piglet intestinal tissue after oral challenge with bacteria[a]

Piglet no.	Small intestine	Large intestine
C. jejuni infected		
1	−	+ (H, P)
2	+ (H)	+ (F)
3	−	+ (D)
4	+ (H)	+ (H, M, F)
5	+ (E)	+ (H, E, M)
6	+ (E, M)	+ (E, M)
7	+ (H, E, M)	+ (E, M)
E. coli infected		
8	−	−
9	−	−
10	−	−

[a] −, normal (no changes observed); +, changes observed (observations indicated in parentheses); E, edema; D, distended with gas; F, frank hemorrhage; H, hyperemia; M, mucus; P, petechial hemorrhage.

TABLE 3. Postnecropsy microscopic examination of piglet intestinal tissue after oral challenge with bacteria[a]

Piglet no.	Lesions in[a]:		Immunoperoxidase staining with *Campylobacter*-specific MAb 1B4[b]
	Small intestine	Large intestine	
C. jejuni infected			
1	−	+ (C, R)	−
2	+ (C)	+ (C, D, R)	−
3	+ (N)	−	−
4	+ (N)	+ (D, N, R)	−
5	−	+ (D, N, R)	+
6	+ (D, N, R)	+ (D, N, R)	+
7	+ (N, R)	+ (D, R)	+
E. coli infected			
8	−	−	−
9	−	−	−
10	− (N)	−	−

[a] −, normal (no lesions observed); +, lesions observed (lesions indicated in parentheses); C, congestion of mucosal and serosal blood vessels; D, damage to intestinal surface epithelial cells; N, neutrophils present either in lumen or in lamina propria; R, small rod- or comma-shaped bacteria in crypts or in surface epithelial cells.

[b] −, negative; +, positive.

ticase soy agar (BBL, Cockeysville, Md.) containing 5% citrated bovine blood and spectinomycin (400 μg/ml), and (iii) Butzler medium containing 4% citrated bovine blood. Tergitol 7 agar was incubated aerobically at 37°C and examined for the presence of *Salmonella* spp. Trypticase soy agar plates were incubated anaerobically (50% hydrogen, 50% carbon dioxide) at 42°C and examined for the presence of *Serpulina hyodysenteriae*. Butzler medium was incubated microaerophilically (10% hydrogen, 10% carbon dioxide, 80% nitrogen) at 37°C and examined for the presence of *Campylobacter* spp.

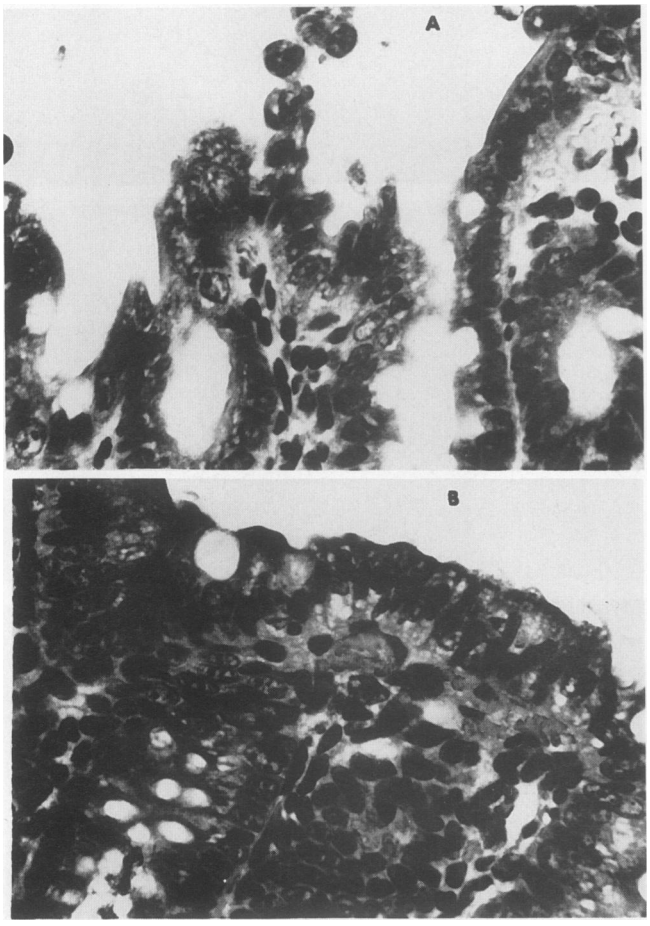

FIG. 2. Light micrograph of large intestine from neonatal piglets. (A) Colon from *C. jejuni*-infected piglet showing damage to epithelial cells; (B) colon from *E. coli*-infected piglet lacking damage to epithelial cells. Stain, hematoxylin and eosin; magnification, ×400.

Necropsy. Piglets were sacrificed 3, 4, and 6 days postinoculation (p.i.) by using 2 ml of Beuthanasia-D (Schering Corp., Kenilworth, N.J.). Postmortem observations of the small intestine, colon, cecum, and other body organs were recorded. Portions of the small intestine, colon, and cecum were fixed in 10% buffered formalin. These fixed tissues were embedded in paraffin, sectioned (6 μm), and stained with hematoxylin and eosin or Warthin-Starry stain and observed by light microscopy.

Electron microscopy. Tissues were immersed in 3.5% glutaraldehyde overnight for fixation. After being washed in 0.1 M cacodylate buffer, the tissues were postfixed with 1% osmium tetroxide for 90 min. After three washes in cacodylate buffer, the tissues were dehydrated in a graded series of ethanol (35 to 100%). For observation under scanning electron microscopy, portions of the tissues were critical point dried in CO_2 and then sputter-coated with palladium-gold (Hummer I; Techniques, Alexandria, Va.). The tissues were examined with a model DS-130 scanning electron microscope from International Scientific Instruments (Milapitas, Calif.).

For observation under transmission electron microscopy (TEM), the remaining tissues were infiltrated and embedded in Epon and then cured at 50, 75, and 100°C for 12, 12, and 1 h, respectively. The sections were then cut with a diamond knife, counterstained with Reynold's lead citrate and uranyl acetate, mounted on copper grids, and examined with either a Hitachi H500 (Scientific Instruments, Mountain View, Calif.) or a Philips CM 12 (Mahwah, N.J.) electron microscope.

Immunoperoxidase staining. Intestinal tissue sections (5 μm) from both principal and control piglets were cut, placed on glued slides, and dried in a 60°C oven overnight. The

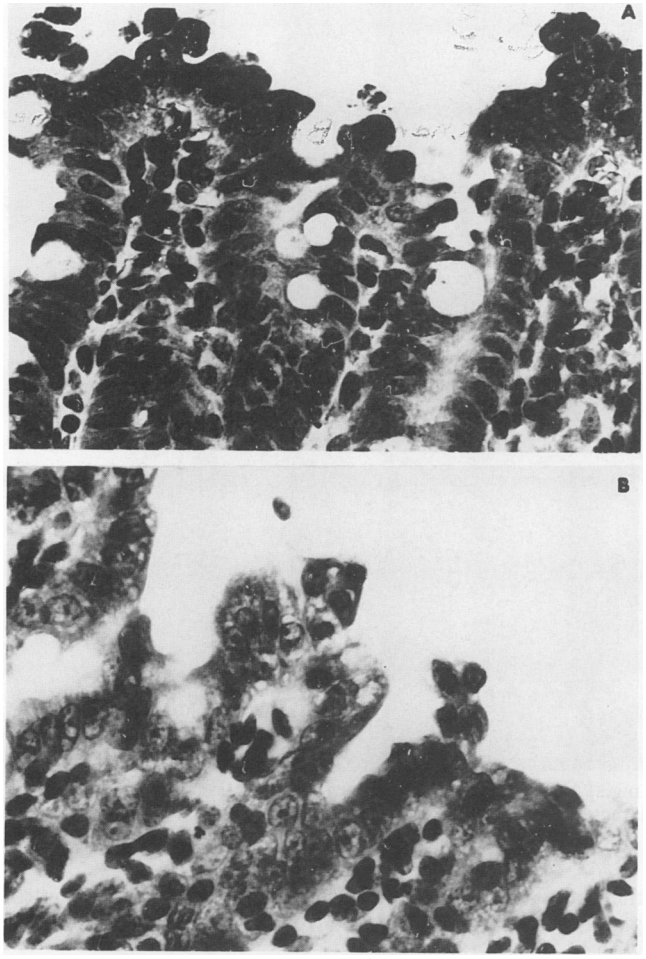

FIG. 3. Immunoperoxidase staining of large intestine from neonatal piglets. (A) The large intestine tissue from *C. jejuni*-infected piglets exhibits staining at surface layers when reacted with 1B4 anti-*C. jejuni* MAb (20). (B) In the absence of MAb 1B4, the large intestine tissue sections from *C. jejuni*-infected piglets did not exhibit any staining. Magnification, ×400.

slides were stained by using a Histostain-SP kit (Zymed Laboratories, San Francisco, Calif.) according to the manufacturer's directions. Briefly, the slides were first deparaffinized with xylene and a series of graded alcohols to water. The slides were then submerged in peroxidase blocking solution. After a 5-min rinse in PBS, the slides were incubated first with serum blocking solution for 20 min and then with anti-*C. jejuni* monoclonal antibody (MAb) 1B4 for 90 min at room temperature in a moist chamber. MAb 1B4 was prepared in-house against surface antigens of *C. jejuni* (20). As a negative control, tissue sections from principal and control piglets were incubated with PBS in the absence of MAb 1B4. After a second wash in PBS, the slides were reacted with biotinylated anti-mouse antibody. Streptavidin-peroxidase was then added for 10 min, the slides were rinsed with PBS, and then the enzyme was detected with a mixture of substrate and chromogen. The slides were rinsed with water and counterstained with hematoxylin and eosin. After a water rinse, the slides were mounted and observed by light microscopy.

RESULTS

Initial experimental inoculation of young piglets (2 to 4 weeks old) with noninvasive *C. jejuni* strains resulted in no clinical signs of *Campylobacter* infection. Thus, the feasibility of an invasive strain as a pathogenic agent in a newborn piglet model was examined. *C. jejuni* M129 was isolated from a patient with clinical signs of campylobacteriosis and shown to invade tissue culture cells at significantly high levels (0.1%) (21, 22). A noninvasive *E. coli* strain, LE392, was used as a control in the inoculation experiments.

Daily observation. In three separate trials, a total of 10 piglets were orally inoculated either with *C. jejuni* M129 (7 piglets) or *E. coli* LE392 (3 piglets). Each trial included both treatment groups, and the animals were sacrificed at 3, 4, or 6 days p.i. (Table 1).

Diarrhea, profuse at times, was observed in the cages of all *C. jejuni*-infected piglets and was characterized by the presence of visible or occult blood and a characteristic slimy texture (Table 1), signs similar to those observed in humans infected with *C. jejuni* (4). After experiencing diarrhea, these piglets became gaunt and restless.

In contrast, the characteristic diarrhea described above was absent from control piglets. Piglet 8 showed no signs of illness. Loose and watery diarrhea was observed in piglets 9 and 10, presumably because of heavy colonization of the intestine with bacteria. The presence of occult blood (but not visible blood) in piglet 10 was judged to be due to overvigorous collection of stool samples. This conclusion is based on the lack of any macroscopic or microscopic lesions in the intestine of this piglet.

Microbiology. Prior to inoculation, rectal swabs from each piglet were cultured for the enteric pathogens of swine, including *Salmonella* spp., *S. hyodysenteriae*, and *Campylobacter* spp., which elicit similar intestinal lesions. None of the above microorganisms was detected in the feces of piglets prior to inoculation. After inoculation, only the principal piglets inoculated were excreting *C. jejuni* in their feces. This excretion was detected at either 24 (piglets 1, 2, 3, 4, and 7) or 48 (piglets 5 and 6) h after inoculation and persisted up to 6 days p.i., albeit in lower numbers (not shown). *C. jejuni*-infected humans are known to excrete the organism in their feces for an average of 2 to 3 weeks (3). Neither *Salmonella* spp. nor *S. hyodysenteriae* was isolated from any of the piglets during the study period.

Macroscopic examination. Grossly, the small intestine from piglets inoculated with *C. jejuni* did not show any significant lesions. Only in a few cases was there a presence of hyperemia or edema with occasional mucus (piglets 2, 5, 6, and 7). By contrast, the large intestine from the piglets inoculated with *C. jejuni* contained gross lesions consisting of hyperemia with patches of petechial hemorrhage early in infection (piglet 1), i.e., 3 days p.i. (Fig. 1A). Later in infection, 6 days p.i., frank hemorrhage was observed in some of the experimental piglets (piglets 2 and 4; Fig. 1B). In other infected piglets (piglets 5, 6, and 7), the large intestine was either hyperemic or edematous and contained increased amount of mucus (Table 2). Piglet 3, which was necropsied 4 days p.i., had moderately distended intestine with gas. Both the large and small intestines of control piglets appeared normal (Fig. 1C). A yellow coloration of intestinal contents due to milk diet was observed, and any manifestation of hemorrhage, edema, or mucus, indicative of disease, in the intestinal tissues of the control piglets was absent. These results are summarized in Table 2.

Light microscopic examination. With one exception, the

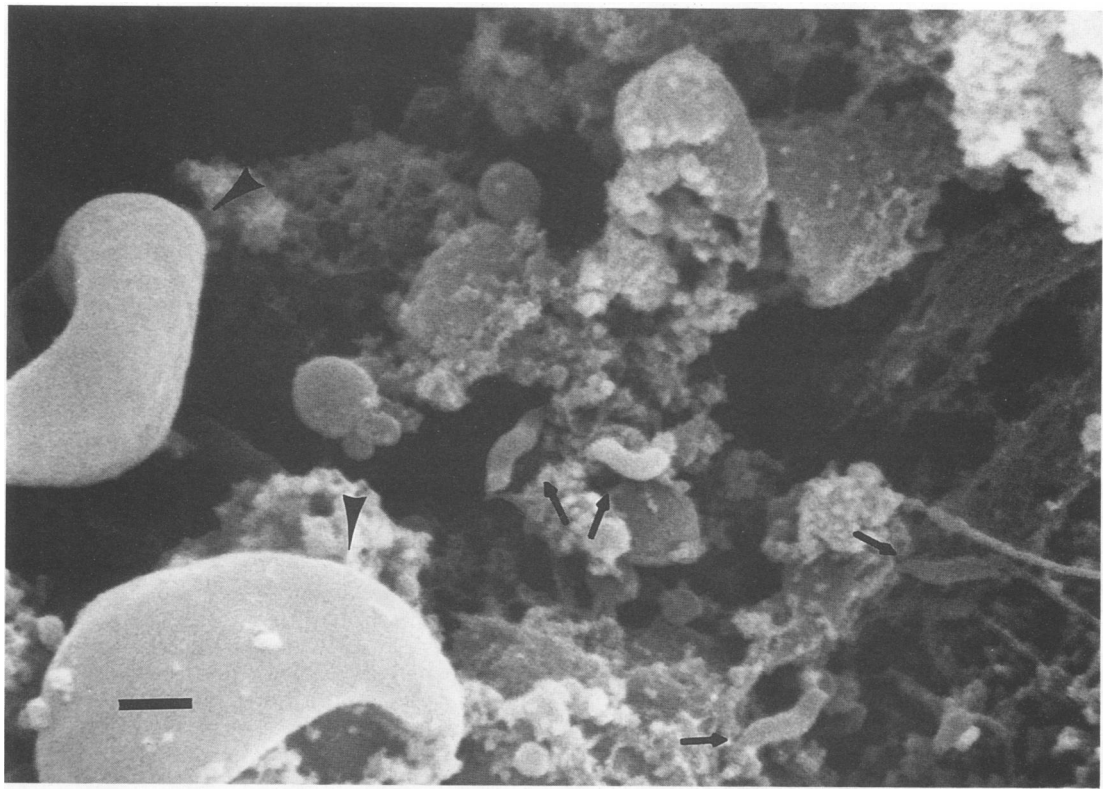

FIG. 4. Scanning electron micrograph of large intestine from neonatal piglet 4 necropsied 6 days after oral inoculation with *C. jejuni*. Note the presence of erythrocytes (arrowheads) and spiral-shaped bacteria characteristic of *Campylobacter* spp. (arrows). Bar, 1 μm.

large intestine proved to be the main site of injury for the piglets inoculated with *C. jejuni*. Significant lesions were apparent only in those *C. jejuni*-infected piglets necropsied 6 days p.i. and consisted of a subacute, diffuse, mild to moderate, erosive colitis and typhlitis. The superficial mucosal epithelial cells exhibited increased cytoplasmic eosinophilia and diminished height (cuboidal rather than columnar) with "rounding up" and exfoliation into the lumen (Fig. 2A). Decreased numbers of mucus-containing cells were seen in this area. The crypts were largely spared, with the exception of pyknotic debris in the crypt epithelium of piglets 4 and 7 (not shown). In three piglets (piglets 4, 5, and 6), small numbers of neutrophils were present either in the lamina propria or as intraluminal aggregates. The lumen and periodically the crypts were heavily colonized by rod-shaped bacteria (piglets 1, 2, 4, 5, 6, and 7). Under high magnification (×1,000), some of these bacteria had a curved-rod morphology characteristic of *C. jejuni* (not shown). These results are summarized in Table 3.

Immunoperoxidase staining with *Campylobacter*-specific MAb 1B4 detected intracellular *C. jejuni* antigens in intestinal tissues of piglets 5, 6, and 7. The MAb 1B4 recognizes outer surface structures of *Campylobacter* spp. (20) and fails to react with other enteric pathogens including *E. coli* and *Salmonella* spp. (not shown). The reactivity of MAb 1B4 with intracellular *C. jejuni* antigen was manifested as a heavy diffuse brown cytoplasmic staining of the superficial mucosal epithelial cells and a light staining at the lamina propria (Fig. 3A). In the absence of primary antibody, the tissue sections from the principal piglets did not show any staining (Fig. 3B), indicating that the staining at the lamina propria and at surface layers most probably is a specific reaction between the antigens and the MAb. The tissue sections from control piglets (piglets 8, 9, and 10) were unstained after reacting with MAb 1B4 (not shown).

Small intestine tissue sections from five of seven experimental piglets also showed some histopathological damage (Table 3). In one case (piglet 6), the damage was characterized by erosion of epithelial cells with exfoliation into the lumen (not shown). In other cases, mucosal and serosal blood vessels were congested (piglets 2 and 6) and neutrophils were present either in the lamina propria, transmigrating between the cells, at the tips of the villi, or in the lumen (piglets 3, 4, 6, and 7; not shown). By contrast, the control piglets had no significant lesions associated with their intestinal tissues. These results are summarized in Table 3.

Electron microscopic evaluation. By using scanning electron microscopy, the large intestine from piglet 4 was compared with that from a control piglet (piglet 8; Fig. 4). The large intestine from the *C. jejuni*-infected piglet was characterized by the presence of heavy amounts of mucus, erythrocytes, and spiral-shaped bacteria characteristic of *Campylobacter* spp. (Fig. 4). By contrast, tissue sections from the large intestine of the control piglet did not reveal any of the above elements (not shown).

The large intestines from experimental and control piglets (piglets 2, 4, and 8) were also compared by using TEM. Whereas cells from control piglet 8 appeared normal, with well-preserved microvillous structures and organized goblet cells, experimental piglets had damaged cells with disrupted microvilli and an excessive amount of mucus (Fig. 5).

Bacteria suggestive of *C. jejuni* M129 were present inside the epithelial cells of experimental piglet 4 but were absent in piglet 8. Although occasionally they were seen attached to

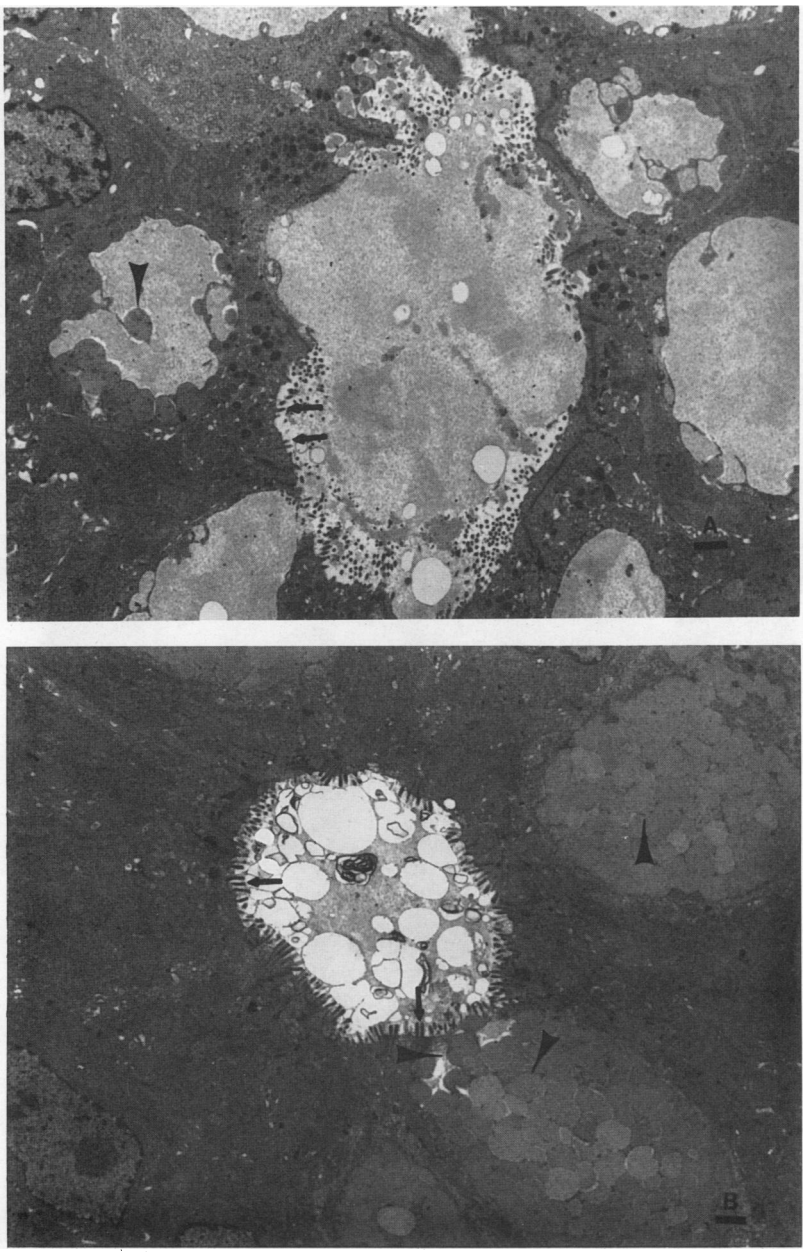

FIG. 5. TEM of large intestine from neonatal piglets 2 and 8 infected with *C. jejuni* (A) or *E. coli* (B). Note loss of microvillous structures (arrows) and mucus depletion of goblet cells (arrowheads) from the intestinal tissues of *C. jejuni*-infected piglets. Microvillous structures and goblet cells appear normal in *E. coli*-infected piglets. Bar, 1 μm.

the microvillous structures (not shown), the majority of these bacteria were detected deep inside the intestinal tissue in the lamina propria (Fig. 6). The surface epithelial cells, in the infected area of the tissue, appeared damaged. This damage was characterized by the replacement of columnar cells with cuboidal cells, in which pyknotic nuclear fragments were present (Fig. 6). Infected cells which contained intracellular bacteria had lost cellular integrity, the endoplasmic reticulum was distorted, and the swollen mitochondria lacked cristae (Fig. 7). It was unclear whether bacteria were inside a vacuole or lying free in the cytoplasm. The majority of bacteria, however, were found in the lamina propria (Fig. 6). The lamina propria also was infiltrated with leukocytes (Fig. 6).

DISCUSSION

Enterocolitis in humans infected with *C. jejuni* has been characterized by the presence of edema, hyperemia, and loss of mucus in both the small and large intestines (10, 23). A nonspecific pathological picture has been seen in rectal biopsy specimens from *C. jejuni*-infected persons (3). Predominant features include ulceration of the mucosal epithelium and loss of mucus. In some of the severe cases, there is distortion of crypt architecture and marked crypt abscess formation in which the lamina propria is infiltrated with neutrophils, mononuclear cells, and eosinophils (10). Attempts to experimentally reproduce the above symptoms in animals have not been fruitful. Only in limited cases with

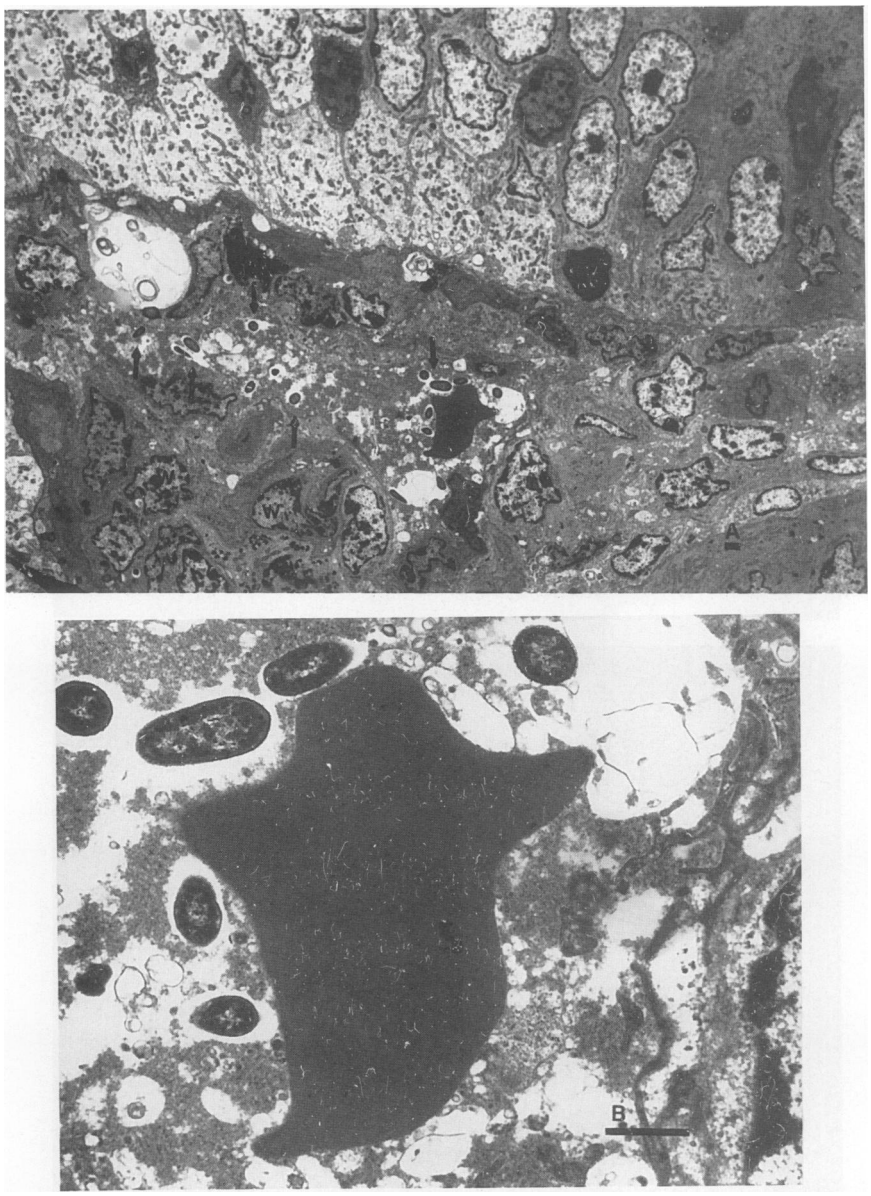

FIG. 6. TEM showing damaged large intestine from piglet 4 infected 6 days earlier with *C. jejuni*. (A) Bacteria (arrow) and leukocytes (W) are present in the lamina propria. P, pyknotic nuclear fragments. (B) Bacteria of panel A (arrows) at a higher magnification. Bar, 1 μm.

prior animal passage of the organism or with prior surgical, chemical, or genetic (i.e., with athymic mice) manipulations of the animals have mild lesions mimicking those in human campylobacteriosis been observed (16, 18, 33). In the present study, oral inoculation of newborn piglets with an invasive clinical strain of *C. jejuni* resulted in clinical and histopathological damage similar to that observed in humans infected with *C. jejuni*. Diarrhea in experimental piglets was characterized by the presence of mucus and blood, as has been noted for humans with *C. jejuni* infections (10, 23). These results are consistent with the findings of Taylor and Olumunmi (31), who reported the presence of intestinal lesions in piglets naturally infected with *Campylobacter* spp., indicating the potential of colostrum-deprived newborn piglets as models for human campylobacteriosis.

The main site of tissue damage appeared to be the large intestine. The damage was characterized by the presence of edema, hyperemia, and mucus depletion. The histological lesions were best demonstrated at 6 days p.i. and included loss of intestinal mucus along with damage to surface epithelial cells. Similar histological findings have been noted for humans with *C. jejuni* infections (10, 23). Although crypt abscess formation was not the salient feature in this model, congestion of mucosal and serosal blood vessels along with the presence of intermittent neutrophil aggregations were occasionally observed in *Campylobacter*-infected tissue sections.

In vitro tissue culture studies indicate that *C. jejuni* is internalized through a process in which the presence of viable bacteria and host cell microfilament functions are required (9, 21). This is a process similar to one observed with some of the invasive enteric bacteria such as *Salmo-*

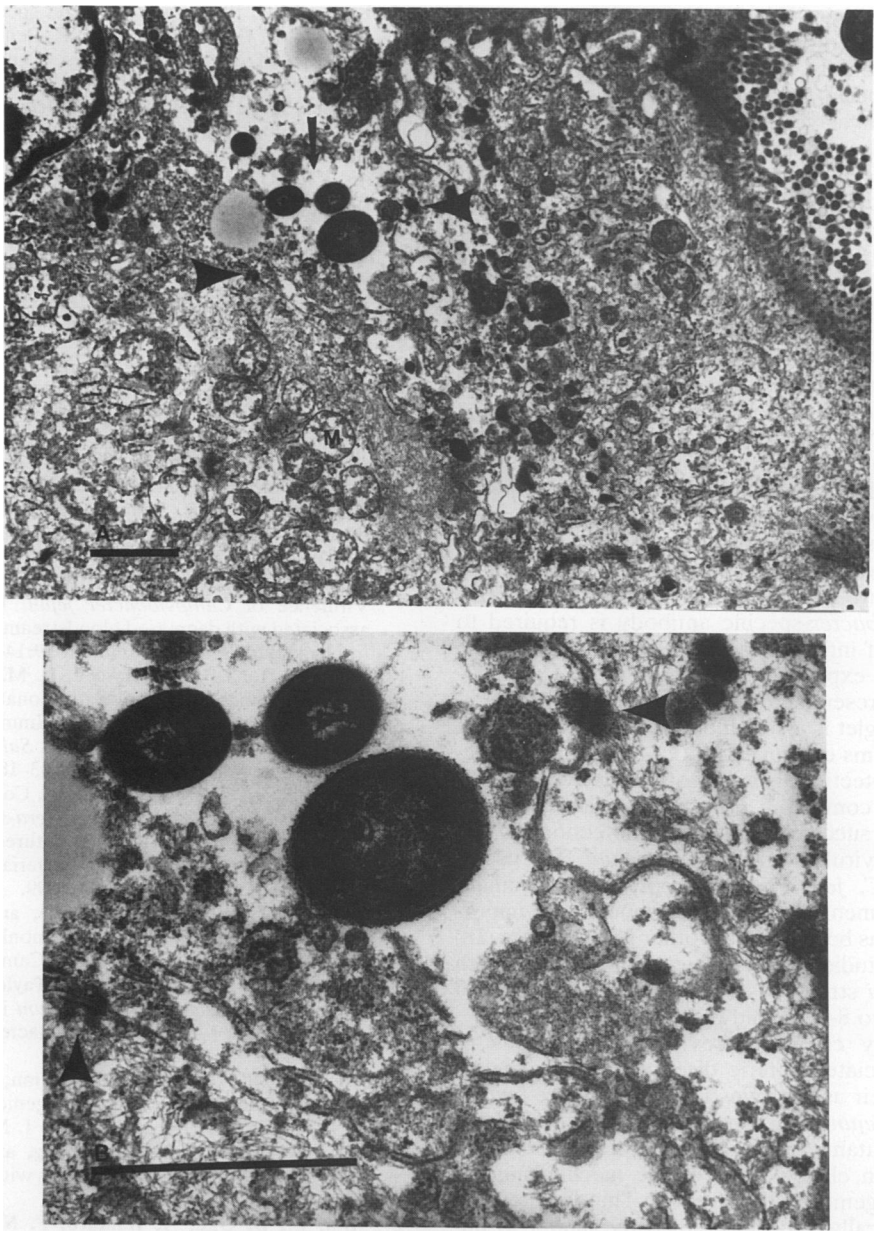

FIG. 7. (A) TEM of large intestine from neonatal piglet 4 infected with *C. jejuni* shows the presence of internalized bacteria (arrow) and swollen mitochondria (M). (B) The locations of desmosomes are indicated by arrowheads at a higher magnification. Bar, 1 μm.

nella spp. (13). In the present study, internalized bacteria were observed only within the intestinal cells of *C. jejuni*-infected piglets, which were accompanied by disruption of microvilli in the infected area. This disruption has also been observed during internalization of other enteric organisms (7, 13). Finlay and Falkow stated that microvillous disruption may be attributed to a rearrangement of actin filaments as seen during the *Salmonella*-induced phagocytosis process (14). Whether the disruption of microvilli in the intestine of *C. jejuni*-infected piglets is the result of microfilament function initiated by *Campylobacter* bacteria needs further investigation. A time course TEM study, involving examination of tissue sections from piglets at different times p.i., could reveal the steps involved in *C. jejuni* internalization after colonization. This type of study may provide further support for the direct tissue invasion concept as opposed to the notion of chemical interactions between the mucosal epithelium and toxic products of *C. jejuni*.

The present study provides indirect evidence for the presence of internalized bacteria in the intestinal tissue of *C. jejuni*-infected piglets. Transmission electron microscopic work revealed the presence of *Campylobacter*-like bacteria in the lamina propria of intestinal tissue from piglets 2 and 4. Immunoperoxidase staining of tissue sections with *Campylobacter*-specific MAb 1B4 revealed the presence of intracellular *C. jejuni* antigens in the intestinal mucosal surface layers in piglets 5, 6, and 7. The lack of MAb 1B4 reactivity with tissue sections from piglets 1 and 3 is consistent with the lack of significant lesions in these piglets that were sacrificed at an earlier stage after inoculation. The lack of

MAb 1B4 reactivity with intestinal mucosal surface layers of piglets 2 and 4 may be due to the distribution of epitopes recognized by this antibody. Previously, it was reported that MAb 1B4 heavily labeled the flagella and unevenly labeled the cell surfaces of invasive *C. jejuni* strains (20). In addition, easily detached capsular material was also shown to react with MAb 1B4 (20). It is possible that at the mucosal surface layers, bacteria as well as detached capsular material or sheared flagella have been phagocytized and therefore have reacted with MAb 1B4, appearing as heavily stained tissue in piglets 5, 6, and 7. In contrast, in piglets 2 and 4, bacteria with uneven distribution of MAb 1B4-specific epitopes have penetrated into deeper layers. With lower concentrations of MAb 1B4-specific epitopes present in the lamina propria, the immunoperoxidase staining may not be sensitive enough to detect the low concentration of antigens in the lamina propria. Supportive of the internalization argument is previous work done in our laboratory with primary swine intestinal cells (unpublished data). *C. jejuni* was able to invade swine intestinal cells at significantly high numbers. In addition, TEM studies by Konkel et al. have demonstrated internalization of *C. jejuni* M129 into human polarized tissue culture cells (22). Additional immuno-TEM work with *Campylobacter*-specific antibody is required to confirm the nature of intracellular bacteria observed in the intestinal cells of the experimental piglets.

In summary, the present study indicates that a colostrum-deprived newborn piglet is a suitable model for studying the pathogenic mechanisms employed by *C. jejuni* during infection. The lack of protection from colostrum-derived anti-*C. jejuni* antibody and competing normal flora facilitates the establishment of a successful intestinal infection in the newborn piglet with virulent strains of *C. jejuni*. The use of virulent strains of *C. jejuni* is an important determinant factor in the development of a successful model. The importance of this factor has been noted by other investigators (18) and in our earlier studies. Previous experimentation with noninvasive *C. jejuni* strains resulted in no clinical signs in orally inoculated 6- to 8-week old piglets.

The present study confirms previous observations of *Campylobacter*-associated enteric disease in young piglets (31) and supports their use in investigating virulence factors associated with *C. jejuni*. The application of infecting this host with defined mutant strains, such as those deficient in invasin antigens, can clarify some of the mechanisms involved in the pathogenicity of *C. jejuni*. This inexpensive host, being anatomically and physiologically similar to humans, should also allow for further examination of the host immune response and the development of potential vaccines in treating severe cases of human campylobacteriosis. This can be achieved in piglets by passive protection of antibody, elicited in dams by a vaccine preparation, or possibly by using a MAb prepared against proteins involved in the invasion process.

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