EXPERIMENTAL GASTROENTERITIS IN NEWLY-HATCHED CHICKS INFECTED WITH *CAMPYLOBACTER JEJUNI*

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SUMMARY. The susceptibility of chicks to enteritis caused by *Campylobacter jejuni* was studied. Three-day-old chicks did not develop enteritis after oral infection but chicks infected within 12 h of hatching developed gastroenteritis. The incubation period correlated with the inoculum size. Initially, infected chicks developed blood- and mucus-containing stools, although watery diarrhoea often occurred late in the course of the disease. Recurrences of the enteric manifestations were common but only two out of 170 infected chicks died. *C. jejuni* was recovered from sites throughout the intestine; the highest concentrations were present in the caecum and large intestine. Both the upper and lower gastrointestinal tract were affected and cellular infiltration of the gastric mucosa and the intestinal lamina propria was observed. Organisms resembling *C. jejuni* were seen within the intestinal epithelium and lamina propria by electronmicroscopy. The newly hatched chick provides a reproducible and sensitive model of campylobacter enteritis.

INTRODUCTION

*Campylobacter jejuni* is a major bacterial cause of acute enteritis in man (Butzler and Skirrow, 1979; Karmali and Fleming, 1979; Prescott and Munroe, 1982). Nevertheless, little is known about its pathogenesis and an animal model of campylobacter enteritis is required for research on the mechanisms of disease. Several reports have described experimental campylobacter infections in several laboratory animals that include mice (Madge, 1980; Field *et al.*, 1981; Welkos, 1982; Blaser *et al.*, 1983), monkeys (Fitzgeorge, Baskerville and Lander, 1981), dogs (Prescott, Manninen and Barker, 1982), chicks (Butzler and Skirrow, 1979; Ruiz-Palacios, Escamilla and Torres, 1981; Manninen, Prescott and Dohoo, 1982; Prescott *et al.*, 1982; Soerjadi, Snoeyenbos and Weinack, 1982), and rabbits (Field *et al.*, 1981; Caldwell *et al.*, 1983). In most animal models, oral infection with *C. jejuni* failed to induce symptomatic enteritis, although reduced carbohydrate absorption in adult mice (Madge, 1980), persistent faecal excretion of the organisms in mice and guinea pigs (Welkos, 1982 and...
unpublished data), low mortality rates of neonatal mice (Field et al., 1981) and microbial invasion of the intestinal mucosa of 8-day-old chicks (Butzler and Skirrow, 1979) have been observed.

Recently, it has been reported that 3-day old chicks infected with *C. jejuni* developed diarrhoea (Ruiz-Palacios et al., 1981). However, other workers have not been able to reproduce enteric disease in 3-day-old chicks fed *Campylobacter* (Manninen et al., 1982). Subsequently, we found that chicks given *C. jejuni* within a few hours of hatching consistently develop enteritis. In this report, the gastrointestinal infection of newly hatched chicks with *C. jejuni* is described.

**Materials and methods**

**Bacterial Strains.** *Campylobacter jejuni* strains A.J. and E.L. were cultured from the diarrhoeal stools of a 4-month-old male and a 20-year old male. *C. jejuni* strain Ch-1 was isolated from chicken faeces collected from a local poultry producer. After primary isolation on a selective agar medium (Campy-BAP, Baltimore Biological Laboratories, Cockeysville, MD), colonies of *Campylobacter* were streaked once on Brucella agar (Gibco Diagnostics, Madison, WI) and stored in Brucella broth (Gibco) with glycerol at −70°C. Isolates were identified according to published procedures (Butzler and Skirrow, 1979; Skirrow and Benjamin, 1980a and b). Additional biochemical reactions and antibiotic sensitivity tests were done to verify the identity of each strain after recovery from experimentally-infected chickens (Skirrow and Benjamin, 1980a and b; Welkos, 1982a).

**Embryonated hens’ eggs** were purchased from a commercial hatchery (Clay’s Hatchery, Blackstone, VA) and were produced by a stock of DeKalb X-L leghorn chickens. This flock was verified as free from *Salmonella pullorum* and *S. typhi* infection and to have no detectable viral pathogens. The eggs were incubated in an Automatic Roll-X Incubator (Marsh Farms, Garden Grove, CA) in standard conditions (National Research Council, 1966) and chicks were removed from the incubator as soon as possible after hatching. They were placed in sterilized plastic cages with raised wire bottoms, four chicks in each cage except where otherwise noted, and the cages were covered with filter tops. Inocula of either *C. jejuni* (120 chicks) or sterile broth (58 chicks) were administered to the chicks within 2–12 h of hatching. At 48 h of age, the chicks were given water and antibiotic-free feed (Purina chick chow, S-G 5065) *ad libitum*.

Fifty-one one-day old chicks obtained from Clay’s Hatchery, were given similar inocula on the first day (26 chicks) or third day (25 chicks) after hatching.

(ii) **Cloaca1 cultures.** Immediately before inoculation of *C. jejuni* cultures or control broth, the cloacae were swabbed and the cloacal contents streaked on sheep-blood-agar plates (BAP) (Tidewater Scott, Chesapeake, VA), MacConkey agar (Difco Laboratories, Detroit, MI) and a medium for isolation of *C. jejuni* consisting of Brucella agar supplemented with five antimicrobial agents (Blaser et al., 1980) and the reducing agents described by George et al. (1978). The BAPs were incubated at 37°C in a candle jar, the MacConkey plates at 37°C in air, and the campylobacter medium at 42°C in microaerophilic conditions (Skirrow and Benjamin, 1980b; Welkos, 1982). Low numbers of aerobic bacteria were detected in 11 out of 76 (14.5%) of the newly hatched chicks. The remainder had no detectable aerobic flora and none of the birds had cloacal cultures positive for *C. jejuni*. Also, no *Campylobacter*-like organisms were isolated from the contents of 40 fertile eggs cultured after incubation for 8–18 days. In contrast to the newly-hatched chicks, 32 of 36 (89%) one-day-old chicks had cloacal cultures positive for aerobes, primarily *Escherichia coli*, enterococci, and *Proteus mirabilis*; however, *C. jejuni* was not isolated from any of these birds.

**Preparation and administration of the bacterial inocula.** Before each experiment, a vial of *C. jejuni* was thawed, streaked on Brucella agar and incubated at 42°C in microaerophilic conditions. Colonies were suspended in Brucella broth and the concentration of organisms estimated spectrophotometrically and confirmed by plate viable counts. A suspension containing 1 × 10⁶ cfu/ml had an absorbance of 0.25 at 590 nm (Coleman-Thomas system 720,
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A. H. Thomas Co., Philadelphia, PA). Age-matched chicks were given oral inocula containing various doses of C. jejuni or sterile broth. Each inoculum (0.3 ml) was delivered to the crop with a 1-ml syringe attached to a 1/2-in, 20 gauge ball-tipped animal feeding needle (Popper and Sons, Inc., New Hyde Park, NY).

Clinical manifestations. Birds were observed daily for signs of illness. Infected chicks were frequently lethargic and showed a decreased rate of weight gain in comparison with uninfected chicks but these changes were not consistent. Fresh stools from each infected chick were examined at intervals from 6 h to 14 days after inoculation for comparison with stools of uninfected controls. Faecal material was smeared and stained directly with Wright's stain (MCB Manufacturing Chemists Inc., Cincinnati, OH) for detection of blood cells. Based on clinical observations, the manifestations of C. jejuni infection were classified as (i) severe enteritis: grossly bloody, mucus-containing stools or watery-to-liquid stools with excess quantities of mucus or gas compared to controls; (ii) mild enteritis: less severe signs of disease, i.e., liquid stools or stools containing visible but trace amounts of blood and mucus; and (iii) asymptomatic infection: stools similar to those of uninfected control birds.

Necropsy and gross pathology. Infected and age-matched control chicks were killed by ether anaesthesia from 6 h to 14 days after inoculation. The gastrointestinal tract was removed and kept on ice. Gross pathological changes were observed with the aid of a hand-held lamp with a magnifying glass (Spectroline Q-225, Spectronics Corp., Westbury, NY) and small sections of the intestinal tract were removed for other procedures.

Bacteriology. Blood for culture was obtained by heart puncture. Brucella agar plates and semi-solid broth were inoculated and incubated for recovery of C. jejuni (Welkos, 1982). Stools and small segments (1–2 cm) of the gastrointestinal tract were cultured on the campylobacter medium. For quantitative recovery of C. jejuni, these specimens were homogenised, diluted in Brucella broth and plated on the campylobacter medium as described previously (Miles, Misra and Irwin, 1938; Welkos, 1982).

Histology and fluorescence microscopy. Cross-sections (2–4 mm) of the gastrointestinal tract were collected and prepared for staining with Haematoxylin and Eosin or Alcian blue or by the indirect fluorescent antibody (IFA) method (Luna, 1968; Kawamura, 1977; Turnbull and Richmond, 1978). For the latter procedure, New Zealand White rabbits (Beechwood Rabbitry, Claremont, VA) were given intravenous injections of a formalin-treated suspension of C. jejuni stain A.J., as described previously (Lior, et al., 1982). The antiserum had a titre of 1600, determined by tube agglutination (Lior et al., 1982). Tissue sections were exposed for 2 h at 37°C to homologous rabbit antiserum diluted 1 in 400 followed by incubation for 1 h at 37°C with a 1 in 20 dilution of goat anti-rabbit globulin conjugated with fluorescein isothiocyanate (Cappel Laboratories, West Chester, PA). In control tests, tissues from infected and uninfected chickens were incubated with PBS, pre-immune rabbit serum, or antiserum absorbed with the C. jejuni antigen. Preparations were viewed and photographed with an Olympus epifluorescent ultraviolet microscope equipped with an Olympus model PM-10 AD photomicrographic system.

Electron microscopy. Gastrointestinal tissues were fixed for 3 h in 0.1 M cacodylate buffer containing glutaraldehyde 2.5% and paraformaldehyde 2%o, pH 7.4. All buffer reagents were purchased from EM Sciences, Fort Washington, PA. The tissues were post-fixed in 1% osmium tetroxide, dehydrated and prepared by standard procedures for transmission or scanning electronmicroscopy (EM) (Dawes, 1971). The samples were examined with a Jeol (JSM-35) scanning electronmicroscope and a Philips 301 transmission electronmicroscope.

RESULTS

Campylobacter infection of one- and three-day-old chicks

None of the 3-day old chicks infected with C. jejuni strains A.J. or Ch-1 developed gastroenteritis or appeared different from the uninfected controls. The inoculated strains were excreted in the stools of these chicks throughout the experimental period. In contrast, five of 16 one-day-old chicks given either C. jejuni strain A.J.
(3 out of 8) or E.L. (2 out of 8) developed enteric symptoms. All chicks survived the infection which was characterised primarily by the presence of blood and mucus in the stool. The onset of the short-lived symptoms occurred 2–5 days after inoculation and by 2 weeks after inoculation the birds had recovered. *C. jejuni* could be recovered from the stools of all infected chicks throughout the period of study. Several chicks were killed 2 weeks after inoculation to culture the gastrointestinal tract. *C. jejuni* was recovered from sites from the crop to the ileum, but the counts were \( \leq 10^5 \text{ cfu/g.} \) Much higher concentrations of *C. jejuni* were recovered from the large intestine and, especially, the caecum, which had a mean *C. jejuni* count of \( 4.9 \times 10^8 \text{ cfu.} \)

**Campylobacter infection of newly hatched chicks**

**Manifestations of enteritis.** Newly hatched chicks appeared to be significantly more susceptible than the older chicks to overt disease. As illustrated in fig. 1, enteritis occurred within 3 days after inoculation in >95% of newly hatched chicks infected with \( \geq 10^7 \text{ cfu of C. jejuni.} \) The onset of illness in chicks infected with the largest dose (3 \( \times \) 10^9 cfu) was rapid and the mean incubation period was 12 h. With smaller doses of *C. jejuni*, the incubation period increased and the attack rate declined slightly. The minimal infectious dose was not determined; however, 30 cfu induced enteric symptoms within 4 days in >50% of the infected chicks. The enteritis was rarely fatal; only 2 of 120 infected chicks and none of the controls died during the course of the experiments (this difference was not significant).

Fig. 2 depicts the gastrointestinal manifestations in chickens infected with the

![Graph](image-url)
Days after inoculation

Fig. 2.—Clinical manifestations in chicks given $3 \times 10^9$ cfu of *C. jejuni*; stools produced by each chick were observed at intervals from 6 h to 14 days after inoculation. ■—Blood and mucus in the stool; □—liquid-to-watery stools, often with excess mucus or gas; ☐—both blood and mucus and liquid-to-watery stools. The data presented are combined results of 44 chicks in five batches. Chicks infected with different inocula exhibited similar clinical features.

largest dose of *C. jejuni*. From 6 h to 1½ weeks after inoculation, the major sign of disease was the presence of blood and mucus in the stool. The stools were formed but were grossly bloody and mucoid or contained strands of blood-tinged mucus. Stained smears of these stools revealed red blood cells and mononuclear white cells. Towards the end of the first week and again 2 weeks after inoculation, chicks commonly excreted diarrhoeal stools or produced liquid stools with blood and mucus. During the course of infection, intermittent recurrences of the exudative or diarrhoeal manifestations or both were commonly observed. The course of the disease was similar in chicks given smaller inocula.

**Bacteriology of infected chicks.** Chicks given at least 30 cfu of *C. jejuni* eventually excreted the organism in the stool. The time after inoculation when *C. jejuni* was first detected in the stool correlated with the inoculum size. Each chick given $\geq 10^7$ cfu of *C. jejuni* had a positive stool culture by 6 h after infection. Nearly 75% of the chicks given 30 cfu had positive stool cultures by 3 days after inoculation, although the rest of these birds did not excrete *C. jejuni* until 9–12 days after inoculation. Once excretion of *C. jejuni* was detected in a chick, the faecal cultures remained positive for the duration of the experiment.

Fig. 3 shows the changes in the number of *C. jejuni* recovered from the stools of infected chicks. When chicks were infected with $3 \times 10^7$ cfu of *C. jejuni*, the numbers of organisms found in the faeces increased up to 2 days after inoculation (fig. 3A). This high concentration of *C. jejuni* was maintained for the 14-day period. The number of organisms excreted in the stool of chicks given the higher dose reached a peak by 2 days after inoculation (fig. 3B). In most of these chicks, the stool *C. jejuni* counts then gradually declined. However, in one experiment, a ten-fold increase occurred in the
14-day stool culture in comparison with the 7-day stool culture (data not shown). This increase was associated with a recurrence of severe enteritis in these chicks.

Quantitative cultures of the gastrointestinal tract from the crop to the large intestine were done in one group of infected chicks (table). To minimise reinfection, the chicks were housed in individual cages, food and water were replaced daily and the

**TABLE**

*Isolation of C. jejuni from the gastrointestinal tract of chicks after oral infection*

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Chick no.</th>
<th>Crop</th>
<th>Gizzard</th>
<th>Duodenum</th>
<th>Mid-gut</th>
<th>Ileum</th>
<th>Caecum</th>
<th>Large intestine</th>
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<td>3.8</td>
<td>5.4</td>
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* No *C. jejuni* isolated; value indicates lower limit of detection for the specimen.
cages were cleaned frequently. Throughout the 2-week period, the largest numbers of *C. jejuni* were present in the caecum and, to a less extent, in the large intestine. Except for the transient high numbers of *C. jejuni* in the ileum, the organisms were recovered in much smaller numbers from the rest of the gastrointestinal tract. *C. jejuni* appeared to persist in the gastrointestinal tract, at least during the period of study.

**Fig. 4.**—Histological changes in the gizzard mucosa of infected chicks: (A) cellular infiltration of the epithelium; (B) sloughing of mucosal cells into the outer membrane of the gizzard; (C) typical gizzard mucosa of uninfected control chick. Ep = gastric epithelium; OM = secreted outer membrane; LP = lamina propria; shows accumulation of erythrocytes, mononuclear cells and eosinophils. Haematoxylin and Eosin (× 87).
Blood cultures from the chicks described in the table were negative for *C. jejuni*. In a separate experiment, cultures of specimens collected from blood, gall bladder, liver, and spleen of chicks given a higher dose ($3 \times 10^9$ cfu) were positive for *C. jejuni* on days one and two after inoculation but were negative thereafter.

**Gastrointestinal pathology.** By 6 h after inoculation of $3 \times 10^9$ cfu of *C. jejuni*, excess mucus and gas appeared in the upper gastrointestinal tract and watery fluid accumulated in the lumen of the small intestine. These signs were observed again on days 3 and 5 after inoculation in the small intestine and in the caecum. Blood and mucus were often seen in the lumen of the small intestine of chicks 12 h after inoculation. The blood and mucus exudate was especially prominent 24 h after inoculation and it decreased in volume and frequency on subsequent days. This luminal exudate was present from the distal portion of the duodenal loop to the terminal ileum and in the large intestine. The gizzard mucosa of chicks studied throughout the 5-day period had a patchy erythema, especially on days 2–5. Petechial haemorrhages of the mucosa were sometimes seen although gross ulceration was observed only rarely. No overt pathology was observed in single chicks examined on days 7 and 14 after inoculation. Chickens given $3 \times 10^7$ cfu of *C. jejuni* had similar early pathological changes.

Several histological changes were observed in the gastrointestinal tracts of infected chicks. A marked cellular accumulation occurred in the lining cells and the secreted luminal membrane of the gizzard mucosa in chicks studied from 6–72 h after

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Fig. 5.—Histological changes in the large intestinal mucosa of infected chicks: (A) submucosal oedema (▲) in the caecum 24 h after inoculation, H & E (× 55); (B) large intestinal mucosa of infected chick which appears flatter and broader than (C) mucosa of control chick, H & E (× 69).
inoculation (fig. 4). An increased number of sloughed mucosal cells was observed in the secreted membrane, and an infiltrate of mononuclear cells, erythrocytes, and granulocytes was noted adjacent to, or transversing, the epithelial border and within the lamina propria. Overt haemorrhage was detected occasionally (fig. 4A). Moderate infiltration of these cell types was also observed in the intestinal lamina propria, especially in the mucosa of the lower small intestine, caecum and colon. Lumen mucus was detected by staining with Alcian blue, and blood cells (erythrocytes, mononuclear cells, and, sometimes, polymorphonuclear cells) were present in small and large intestinal exudates. Mucosal and submucosal oedema was observed throughout the gastrointestinal tract, most frequently in the caecum (fig. 5A). In specimens of the lower gastrointestinal tract taken 48-72 h after inoculation, the mucosa was often hyperplastic and the epithelial border was flattened and broadened when compared to controls (figs 5B and C). Overt disruption of the intestinal mucosa was not observed microscopically except for apparent necrosis of the gizzard epithelium detected by EM in infected chickens studied 6 h after inoculation.

**EM and fluorescence microscopy.** During the first 24 h, *C. jejuni* was observed by EM in the lumen throughout the gastrointestinal tract. They were most numerous
Fig. 7.—Transmission electronmicrographs showing *Campylobacter*-like organisms (▶) within gastrointestinal epithelial cells and in the junctional spaces between cells from infected chicks: (A) mid-gut epithelium containing numerous inclusion bodies and probable membrane-bound organisms (×3400); (B) higher magnification (×12,750) of bacterial inclusion from (A); similar inclusions between the intercellular membranes of (C) ileal epithelial cells (×12,750) and (D) caecal epithelial cells (×9350), and (E) in the basal portion of the cell cytoplasm (×2890; inset, ×21,250).
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Fig. 8.—Transmission electronmicrographs showing Campylobacter-like bacteria (*) in the caecal mucosa of an infected chick 24 h after inoculation: (A) in the lumen and within epithelial vacuoles (× 2890); (B) and (C) within and outside cells of the lamina propria (× 2125 in B and 6035 in C). RBC = red blood cell in the lamina propria.

within the secreted luminal membrane of the gizzard mucosa and in the lumen of the ileum, caecum and colon (fig. 6). The organisms were observed near both the apical and basal portions of the intestinal mucosal fold but they were most frequent in the basal crypt-like areas. Frequently the organisms appeared to be in direct contact with either the intestinal glycocalyx or the epithelial cell membrane, especially at

Fig. 9.—Indirect immunofluorescent staining, with rabbit antiserum prepared against C. jejuni strain A.J. and FITC-labelled goat anti-rabbit globulin, of chick large intestinal mucosal tissue. (A) Mucosa from an infected chick 12 h after inoculation showing fluorescent cell within the lamina propria; (B) uninfected tissues incubated with antiserum showing background fluorescence (× 577).
intercellular junctions. In addition, bacteria were detected within intestinal epithelial cells and in the lamina propria of specimens collected 6–24 h after inoculation. The bacteria were present between the intercellular membranes as well as in membrane-bound cytoplasmic vesicles (fig. 7). Most of the organisms appeared to be structurally intact. *C. jejuni*-like bacteria were observed below the epithelial cell layer, both within and outside of cells of the lamina propria. In several instances, a single tissue sample contained *C. jejuni* in all of the locations described above (fig. 8). Bacteria and inclusions similar to those observed in *C. jejuni*-infected chicks were not seen in tissues collected from uninfected chicks.

The results of IFA staining of intestinal epithelial tissues supported the EM evidence for penetration of the intestinal mucosa by *C. jejuni*. In tissues taken from the middle small intestine to the colon, organisms in the lumen and within the epithelium and cells of the lamina propria were stained with the antiserum (fig. 9).

**DISCUSSION**

*C. jejuni* is a widespread and major gastrointestinal pathogen of man, but little is known about the pathogenesis of campylobacter enteritis. An animal model of the disease would facilitate studies on bacterial virulence properties and the mechanisms of disease.

Many animal species appear to be healthy intestinal carriers of *C. jejuni* and reservoirs of the infection for man (Butzler and Skirrow, 1979; Prescott and Munroe, 1982; Shanker et al., 1982). A suitable model of campylobacter enteritis would involve animals that are not asymptomatic carriers and that develop signs of disease after oral inoculation similar to those in man. Diarrhoeal disease attributed to *C. jejuni* has been produced in some domestic animals, gnotobiotic dogs, and monkeys (Fitzgeorge et al., 1981; Prescott et al., 1982; Taylor, 1982,) but not in small laboratory animals.

Chickens have also been the subject of numerous investigations on campylobacter infection. *C. jejuni* has been isolated from apparently healthy chickens and from processed poultry; however, it appears that intestinal colonisation is not a uniform occurrence (Prescott and Munroe, 1982; Shanker et al., 1982). *C. jejuni* is not clearly associated with a naturally-occurring clinical syndrome in chickens, although it appears to have been the agent of avian vibrionic hepatitis. In this disease, diarrhoea was a common manifestation (Peckman, 1972). Recently, chickens have been examined as possible models for campylobacter enteritis. The results of several studies have varied. Butzler and Skirrow (1979) administered *C. jejuni* to 8-day-old chicks but no signs of disease were observed although the organisms were recovered by culture and were observed by EM within the caecal wall. Similarly, Prescott et al. (1982) reported that 5-day-old gnotobiotic chicks failed to develop enteritis after infection with *C. jejuni*. In contrast, Ruiz-Palacios et al. (1981) and Soerjadi et al. (1982) have reported the induction of diarrhoea in 3-day-old chicks fed with clinical isolates of *C. jejuni*. However, we and Manninen et al. (1982) failed to produce clinical illness in 3-day-old chicks infected with various strains of *C. jejuni*. The variability in susceptibility may be due to differences in the strains of *C. jejuni* used or in the source and strain of chicks. Chicks appear to have an age-related susceptibility to campylobacter enteritis. Almost one-third of the one-day-old chicks and nearly all of the newly-hatched birds in our study developed enteritis. There are probably several
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reasons for the increased susceptibility of the newly hatched chicks. Young birds lack a fully developed flora, which may play a protective role against enteric pathogens (Abrams and Bishop, 1966; Turnbull and Richmond, 1978; Berg and Garlington, 1979). Also, the immunological, anatomical and physiological immaturity of the intestinal tract and reticuloendothelial system of newly hatched chicks may contribute to increased susceptibility to infection (Shaffer et al., 1964; Abrams and Bishop, 1966; Fuller and Jayne-Williams, 1970; Lawrence et al., 1981).

Oral inoculation of *C. jejuni* into the newly hatched chicks was clearly associated with an enteric clinical syndrome. Similar and different clinical manifestations have been described for the models of chick enteritis reported previously (Ruiz-Palacios et al., 1981; Soerjadi et al., 1982). Both our newly-hatched chicks and the 3-day-old chicks described by Ruiz-Palacios et al. (1981) developed enteric illness after small inocula of *C. jejuni*. Also the incubation periods were similar, a mean of 43 h in our chicks given $3 \times 10^7$ cfu and 45 h for the 3-day-old birds receiving $9 \times 10^7$ cfu. In contrast to the mortality rate of 32% reported by Ruiz-Palacios et al. (1981), campylobacter infection was rarely lethal in our birds. Diarrhoea was the major enteric sign previously observed in chicks (Ruiz-Palacios et al., 1981; Soerjadi et al., 1982) but campylobacter infection of the newly-hatched animals produce predominantly an exudative enteritis and a lower incidence of watery diarrhoea. The histological manifestations of campylobacter enteric infection were generally similar to those reported before (Ruiz-Palacio et al., 1981), i.e., mucosal oedema and mild cellular infiltration of the lamina propria. In addition to these findings, an apparent gastritis was observed in the newly hatched chicks. Although *C. jejuni* is susceptible to killing by acid (Blaser et al., 1980), the gizzard mucosa of the newly hatched chick has a higher pH (5.1–6.2) before exposure to food and water than it does afterwards (3.3–4.5) (Shaffer et al., 1964). The chicks in our study were given *C. jejuni* before feeding. Bacterial infections of the gastric mucosa have been documented for other gastrointestinal pathogens, e.g., mild to severe gastritis in experimental *Salmonella* gastroenteritis and shigellosis of rhesus monkeys (Kent, Formal and LaBrec, 1966; Kent et al., 1967).

Further study is required to define the role of gizzard pathology in the pathogenesis of the exudative gastroenteritis observed in the chickens.

A common feature of campylobacter infection of newborn and young chicks is persistance of *C. jejuni* within the gastrointestinal tract (Prescott et al., 1982; Soerjadi et al., 1982; this study). In the newly hatched chick, a large caecal reservoir and extensive faecal excretion occurred. Persistance of *C. jejuni* in the gastrointestinal tract of chickens may be facilitated by the mucosal adherence and invasiveness of the organism. In the newly hatched birds, close contact between *C. jejuni* and the gastrointestinal mucosal epithelium was shown microscopically. Evidence for intra-epithelial localisation of *C. jejuni* from the middle to lower intestine of infected chickens has been reported from different laboratories (Butzler and Skirrow, 1979; Ruiz-Palacios et al., 1981) and in this study. Ruiz-Palacios et al. (1981) suggested that *C. jejuni* can invade the chicken epithelial cell by disruption of the apical cell surface membrane. Our results suggest that *C. jejuni* may also penetrate epithelial cell membranes, within an endosome or via intercellular junctions, and transverse cells in cytoplasmic vacuoles or within intercellular spaces finally to reach the lamina propria. Intact *Campylobacter* cells were observed in the lamina propria both extracellularly and within membrane-bound inclusions of lamina cells. Similar mechanisms of
invasion have been described in the chick and guinea-pig models of salmonella enteritis (Takeuchi, 1967; Turnbull and Richmond, 1978). However, the precise mechanism of penetration of C. jejuni and the importance of this process in chicken enteritis is unknown. In the gastrointestinal tract of gnotobiotic mice, a non-specific process of bacterial translocation can take place, often without adverse effects (Berg and Garlington, 1979). Although Fuller and Jayne-Williams (1970) cultured bacteria from the livers of 4 of 15 newly hatched chicks, Turnbull and Richmond (1978) detected no gastrointestinal translocation in chicks given E. coli K12.

In any event, evidence from several different sources strongly implicates gastrointestinal invasiveness as a primary virulence property of C. jejuni. The organism has been cultured from blood and other extra-intestinal sites after oral inoculation in chickens, other animals and sometimes man (Butzler and Skirrow, 1979; Soerjadi et al., 1982; Taylor, 1982; Blaser et al., 1983). Moreover, in man, several clinical features of campylobacter enteritis resemble those observed in disease caused by invasive gastrointestinal pathogens such as Shigella and Salmonella spp., i.e., fever, abdominal pain and blood in the stool (Butzler and Skirrow, 1979; Karmali and Fleming, 1979). Also colitis or small intestinal haemorrhage or both have occurred in patients with campylobacter enteritis (Colgan et al., 1980; Michalak et al., 1980). C. jejuni infection of the newly hatched chicks was associated with an extensive gastrointestinal blood and mucus exudate but, unlike disease caused by the classical invasive pathogens, mucosal ulceration and destruction were not usually observed.

In summary, the model described in this study is potentially useful for investigating the pathogenesis of C. jejuni infection in birds and in man. The enteritis that followed infection of newly hatched chicks was similar in several respects to that occurring in man. The infection was generally not lethal and the exudative and diarrhoeal characteristics resembled the campylobacter gastroenteritis described in neonates and children (Karmali and Fleming, 1979; Anders, Lauer and Paisley, 1981). In addition, the recurrence of enteric illness in chickens may provide a model for studying the nature of the relapses reported in some patients (Butzler and Skirrow, 1979). Furthermore, enteritis occurred in chickens after oral inoculation and did not require the pretreatment regimens (e.g., opiates or antibiotics), gnotobiotic rearing, or surgical manipulations that are necessary in some animal models to induce disease with enteric pathogens (Takeuchi, 1967; Manninen et al., 1982; Prescott et al., 1982). Low doses of C. jejuni were effective and the manifestations of infection, especially the production of blood and mucus, were relatively easy to detect. Finally, young chicks are convenient and inexpensive animals for laboratory experiments such as those concerning the relative virulence of different strains of C. jejuni.

This work was supported by grant C6/181/89 from the Diarrheal Diseases Control Program (World Health Organization) and by institutional grant #941–98 from Eastern Virginia Medical School.

I am grateful to J. Thompson for valuable histological assistance; J. Slusser and C. Miekley for excellent electronmicroscopy and helpful discussions; G. Bluemink, M. Dufour and G. Borman for pathology consultations; A. O'Brien, S. Leppla and W. Beisel for valuable discussions; G. Wright for generously providing access to his microscopic equipment; and J. Charity, P. Briley and D. Beall for secretarial assistance.
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