Colonization of Gastrointestinal Tracts of Chicks by 
Campylobacter jejuni
J. T. BEERY, M. B. HUGDAHL,† AND M. P. DOYLE*
Food Research Institute* and Department of Food Microbiology and Toxicology, 
University of Wisconsin–Madison, Madison, Wisconsin 53706
Received 21 April 1988/Accepted 7 July 1988

Bacterial enumeration and histologic examination of organs and tissues of 8-day-old chicks 7 days after 
peroral inoculation with Campylobacter jejuni revealed that the organism colonized primarily the lower 
gastrointestinal tract. The principal sites of localization were the ceca, large intestine, and cloaca, where 
densely packed cells of C. jejuni were observed in mucus within crypts. Examination of C. jejuni-colonized 
crypts by transmission electron microscopy revealed that the campylobacters freely pervaded the lumina 
of crypts without attachment to crypt microvilli. Understanding the mechanism of colonization may lead to 
approaches that will reduce the incidence of C. jejuni carriage by poultry.

Considerable evidence indicates that poultry is a principal vehicle of transmission of Campylobacter jejuni involved in 
human illness. Not only have several studies revealed that many of the serotypes of C. jejuni isolated from poultry are 
also frequently associated with human infection (2, 18, 19, 28, 32), but many epidemiologic studies also have identified 
a strong association between Campylobacter enteritis and eating or handling raw or undercooked chicken (8, 11, 13, 14). Data from a large prospective study to determine the 
role of meats as possible sources of infection leading to Campylobacter enteritis in western Washington State indicated 
that ingestion of contaminated chicken contributed to about half the cases of Campylobacter enteritis in that area (11). A case-control study at the University of Georgia to identify risk factors for Campylobacter enteritis among college students revealed that eating chicken was the principal 
vehicle of transmission for sporadic cases of Campylobacter infection (8). Additionally, several outbreaks of Campylo-
bacter enteritis have been linked to eating chicken or poultry products (7, 17, 26, 27, 33, 36).

Poultry is a well-established reservoir of C. jejuni. The organism is carried in the intestinal tract and excreted in 
feces. The number of C. jejuni in intestinal contents of chickens and turkeys is generally at levels of 10⁴ to 10⁶ CFU/ 
g (1, 10, 25, 29, 41). Surveys of live poultry revealed that a large percentage, often greater than 75%, of birds harbor C. jejuni in their intestinal tracts (10, 29, 31, 35, 41). Poultry carcasses and edible parts are often contaminated with C. jejuni during processing, largely through contact with fecal material (12, 29, 41, 42). Hence, about 30% or more of retail 
poultry is contaminated with C. jejuni (21, 38), often at levels of 10⁴ to 10⁶ CFU/g (11, 12, 20, 29).

Considering the apparent prominent role of poultry as a vehicle in the transmission of Campylobacter enteritis, major 
efforts are needed to reduce the prevalence of C. jejuni among poultry flocks. Understanding the mechanism of 
intestinal colonization may provide critical information for the development of practical means to control colonization of C. jejuni. The purpose of this study is to identify the 
principal sites of localization and the physical means of colonization of C. jejuni within chicks.

MATERIALS AND METHODS

Organism and cultural conditions. C. jejuni 74C (Lior serogroup 2, biotype I), a fecal isolate from a laying hen, was 
used for this study. The culture was prepared by growing the organism in 50 ml of brucella broth (Difco Laboratories, 
Detroit, Mich.) containing 0.3% sodium succinate and 0.01% cysteine hydrochloride in a 250-ml sidearm Erlenmeyer 
flask. The flask was evacuated three times to 50.8 cm of Hg and flushed with a gas mixture of 5% O₂, 10% CO₂, and 85% 
N₂. The broth culture was incubated at 42°C in a gyratory water bath at 150 gyrations per min for 18 h. The culture was 
twice sedimented by centrifugation (6,000 × g, 20 min) and washed in 0.01 M phosphate-buffered saline solution, pH 7.0 
(PBS). The cell concentration was adjusted spectrophotometrically (optical density of 0.23 at 550 nm) to the desired 
number (5.2 × 10⁶ cells per 0.5 ml) with PBS, and cell numbers were confirmed by plate counts on brucella agar.

Antiserum to C. jejuni. Antiserum to C. jejuni 74C was prepared by the procedure described by Lior et al. (24), with 
the exception that the antiserum was not absorbed with antigens of C. jejuni. Titers of the antiserum were deter-
menced by tube titration with formalinized cells of C. jejuni 74C (24) and were 1:200 to 1:400. Slide agglutinations were 
performed on glass slides with the antiserum diluted 1:5 with PBS. Presence or lack of agglutination was determined 30 to 
45 s after a loopful of live bacteria was mixed with 50 μl of antiserum. Autoagglutinability was determined by mixing a 
loopful of bacteria with a drop of PBS. No slide agglutinations were observed with strains of Campylobacter coli, 
Campylobacter fetus subsp. fetus, or C. fetus subsp. veneditis, whereas C. jejuni 74C consistently agglutinated with all 
preparations of antiserum.

Enumeration of C. jejuni in chick organs and tissues. Thirty-six 1-day-old White Leghorn chicks were each dosed 
perorally with 5 × 10⁶ C. jejuni in 0.5 ml of PBS. The inoculum was administered into the crop with a 20-gauge 
curved cannula. Twenty-one control chicks were each given 0.5 ml of PBS by the same route. Chicks were placed one per 
cage in an incubator room at 37°C. At 24-h intervals for 7
days, two to seven chicks were sacrificed by carbon dioxide asphyxiation, selected organs and tissues were removed for histology, and the remaining tissue or intestinal segment was weighed and assayed for C. jejuni. Specimens assayed included spleen, gallbladder (with bile), liver, proximal small intestine, distal small intestine, ceca, and large intestine plus cloaca. Organs and tissues used to determine C. jejuni counts were homogenized with a Polytron tissue homogenizer (Brinkmann Instruments, Inc., Westbury, N.Y.) and diluted in PBS. Duplicate platings were done on Campy BAP (5), and plates were incubated for 48 h at 42°C under microaerobic conditions. Colonies with typical C. jejuni morphology were counted, and representative colonies from plates with the highest dilution were examined microscopically and confirmed by biochemical tests (30).

**Histologic examination of chick tissue.** Tissue samples of proximal small intestine, distal small intestine, ceca, large intestine, and cloaca of inoculated and control chicks used in the study described above were placed into paraformaldehyde-periodeate-lysine fixative at 4°C for 6 h (16). A portion of each segment was rinsed with digitonin (16) and embedded in plastic by the cold glycol methacrylate (JB-4; Polysciences, Inc., Warrington, Pa.) method (6). Tissue blocks were cut into sections (2 μm) which were mounted on slides, stained with azure A (23) and with methylene blue-basic fuchsin (4), and examined microscopically. JB-4 plastic tissue sections of ceca, large intestine, and cloaca for which complementary sections exhibited the presumptive presence of C. jejuni were treated with an immunoperoxidase staining procedure to confirm the presence of C. jejuni. The staining was done by the procedure described by Beery et al. (3), using antiserum (1:50 dilution) raised in rabbits to Formalin-treated cells of C. jejuni 74C. Tissue sections then were examined microscopically.

An additional 28 chicks were examined more extensively for colonization by C. jejuni. The chicks (1 day old) consisted of two groups of 14, with 6 control (each perorally inoculated with 0.5 ml of PBS) and 8 treated (each perorally inoculated with 5 x 10⁸ C. jejuni 74C in 0.5 ml of PBS) chicks per group. All chicks were sacrificed 7 days postinoculation, surgically exposed, and examined for gross pathological abnormalities. Organs and tissues, including heart, liver, gallbladder, spleen, kidneys, and gastrointestinal tract (from the proventriculus to the cloaca), were removed, and representative samples were fixed and prepared for microscopic examination by the procedures described above. Additionally, a portion of each cecum, large intestine, and cloaca was taken for examination by transmission electron microscopy.

Specimens were prepared for transmission electron microscopy by rinsing the tissues in digitonin (16), mincing them into 1- to 1.5-mm-square pieces, and washing them with PBS-10% sucrose and then with PBS. Endogenous peroxidase was inactivated by immersing the tissues in 0.005 M periodic acid for 10 min at room temperature and then in 0.003 M sodium borohydride for 30 min followed by several rinses in PBS. Tissues were then immersed for 45 min in PBS-10% sucrose containing rabbit normal serum, rinsed in PBS-10% sucrose, immersed for 3 h in rabbit antiserum raised to C. jejuni 74C (1:50 dilution), rinsed in PBS-10% sucrose, and finally immersed for 1 h in goat anti-rabbit immunoglobulin conjugated with horseradish peroxidase (1:200 dilution; Sigma Chemical Co., St. Louis, Mo.). After being rinsed in PBS, tissues were immersed for 15 min in Karnovsky complete mixture (9) containing 0.01 M imidazole (39), rinsed in 0.05 M Tris hydrochloride (pH 7.6), fixed for 1 h in 1% osmium tetroxide in PBS, and rinsed in PBS.

### Table 1: Distribution of C. jejuni in chick tissues and feces after peroral inoculation as determined by a culture procedure

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. of C. jejuni per specimen</th>
<th>(a) Min. level of detection (b) per g of specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>&lt; 10⁶ (0.00)</td>
<td>&lt; 10⁶ (0.00)</td>
</tr>
<tr>
<td>Liver</td>
<td>&lt; 10⁶ (0.00)</td>
<td>&lt; 10⁶ (0.00)</td>
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<tr>
<td>Spleen</td>
<td>&lt; 10⁶ (0.00)</td>
<td>&lt; 10⁶ (0.00)</td>
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<tr>
<td>Proventriculus</td>
<td>&lt; 10⁶ (0.00)</td>
<td>&lt; 10⁶ (0.00)</td>
</tr>
<tr>
<td>Ceca</td>
<td>&lt; 10⁶ (0.00)</td>
<td>&lt; 10⁶ (0.00)</td>
</tr>
<tr>
<td>Large intestine</td>
<td>&lt; 10⁶ (0.00)</td>
<td>&lt; 10⁶ (0.00)</td>
</tr>
<tr>
<td>Feces</td>
<td>&lt; 10⁶ (0.00)</td>
<td>&lt; 10⁶ (0.00)</td>
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<tr>
<td>Blood</td>
<td>&lt; 10⁶ (0.00)</td>
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(a) Chicks inoculated with 5 x 10⁸ C. jejuni per chick.
(b) Minimum level of detection was 10⁶ C. jejuni per g of specimen.
The tissues were processed through different concentrations of ethanol and embedded in Durcupan ACM Fluka (Polysciences). Ultrathin sections (60 to 90 nm) were mounted on Parlodion-coated 200-mesh grids, stained with uranyl acetate and lead citrate, and examined by transmission electron microscopy (×1,600 to ×38,000). Appropriate controls were done to verify the accuracy of the immunoperoxidase staining procedure.

RESULTS

The physical response of chicks inoculated perorally with C. jejuni was indistinguishable from that of control animals in that there was no apparent loss of appetite, no reduction in weight gain, no alteration in locomotion, and no diarrhea or respiratory distress. All animals, both inoculated and controls, appeared healthy.

Distribution of C. jejuni in tissues and organs. C. jejuni was recovered from tissues and organs of 23 of 36 (64%) inoculated chicks, but not from control chicks. The principal site of localization was the lower gastrointestinal tract, especially in the ceca, where C. jejuni was often detected at levels of \(10^4\) to \(10^7\) cells per g (Table 1). The organism was recovered most frequently from the ceca (20 of 36 chicks, 55.6%), distal small intestine (14 of 36 chicks, 38.9%), and large intestine plus cloaca (5 of 14 chicks, 35.7%), but only twice from the spleen, once from the gallbladder and blood (cardiac puncture), and not from the liver.

C. jejuni was detected by histologic examination in 17 cecal and 11 cloacal and large intestinal specimens from 18 chicks inoculated with C. jejuni and examined 7 days postinoculation. Interestingly, the organism was detected by microscopic examination in the ceca and cloaca of two chicks, whereas it was recovered by plating procedures from only the cecum of one of the chicks. C. jejuni was not detected by histlogic examination in similar tissues of 15 8-day-old control chicks that were not exposed to the organism. C. jejuni was not detected microscopically in the heart, liver, spleen, gallbladder, kidneys, proventriculus, gizzard, duodenum, or proximal small intestine in any of the chicks (inoculated or control) examined 7 days postinoculation. The organism was occasionally detected by histologic examination in the distal small intestine of chicks inoculated with C. jejuni.

Histopathology. Examination of tissues of the gastrointestinal tract revealed no necrosis or invasion of the epithelium...
or any other major pathological differences between specimens from inoculated or control chicks. However, a mild edema of the lamina propria and submucosa was detected occasionally in the proximal ceca of inoculated chicks. There was no evidence of *C. jejuni*-like cells associated with either the surface or glandular epithelium of any tissues of the gastrointestinal tract. *C. jejuni* cells were observed both morphologically and by an immunoperoxidase staining procedure with *C. jejuni*-specific antiserum in the inoculated chicks, principally in cecal, large intestinal, and cloacal crypts (Fig. 1 and 2) and in the lumina of the ceca, large intestine, and cloaca among fecal material. Between 4 and 35% of cecal crypts of 17 *C. jejuni*-positive chicks were filled with densely packed *C. jejuni* cells. A small percentage (<5%) of cecal crypts of control chicks contained some rod-shaped, cocoid, and/or fusiform bacteria (not *C. jejuni* as indicated by immunoperoxidase staining) but at substantially lower numbers than were observed for *C. jejuni* in crypts of inoculated chicks. Bacteria present in cecal, large intestinal, and cloacal crypts of control chicks were generally at the proximal end of the gland and not in the glandular lumina or the distal portion of the crypts (Fig. 1A). In contrast, *C. jejuni* in inoculated chicks filled cecal, large intestinal, and cloacal crypts from the proximal end to the distal end and were most prominent in crypts in the mid and proximal ceca and the cloaca. Immunoperoxidase staining with antiserum raised to *C. jejuni* 74C confirmed that these bacteria were *C. jejuni* (Fig. 3).

Examination of more than 100 transmission electron microscopy photomicrographs of *C. jejuni*-colonized cecal, large intestinal, and cloacal crypts consistently revealed that the campylobacters pervaded the lumina of crypts and were occasionally in close apposition to the glandular microvilli, but never in direct contact with the microvillus outer membrane (Fig. 2 and 3B). Hence, it appeared that campylobacters colonized crypt mucus without attaching to crypt microvilli.

Additionally, based on the staining properties of crypt mucus treated with the mucoprotein-glycoprotein-specific stains azure A and thionine (23), it appeared that *C. jejuni* had modified, perhaps metabolized, the crypt mucus. The cecal and cloacal mucus in crypts filled with campylobacters consistently stained noticeably lighter than mucus in similar crypts of *C. jejuni*-free control chicks.

**DISCUSSION**

Our observations that *C. jejuni* localizes principally in the ceca, large intestine, and cloaca of chicks are in general agreement with the findings of Soerjjadi et al. (37). Although some investigators (34, 40) have reported the occurrence of *C. jejuni*-induced lesions in the intestinal tracts of young chicks, no such lesions were observed in the intestinal tracts of any of the chicks examined in our study.

An elegant study by Lee et al. (22) revealed that in gnotobiotic mice *C. jejuni* colonizes mucus on the outer surface and deep within the intestinal crypts, with the cecal crypts being preferentially colonized. They observed that campylobacters do not adhere to the intestinal surface but are highly motile and rapidly track along intestinal mucus. We made similar observations in chicks, in which *C. jejuni* appears to colonize mucus preferentially within cecal and cloacal crypts without adherence to the crypt surface.

Preferential attraction of *C. jejuni* to mucus-filled crypts may be explained by the chemotactic behavior of the organism toward mucin, a principal constituent of mucus. Hugdahl et al. (15) determined that *C. jejuni* is chemotactically attracted to mucin and to L-fucose, which is likely the principal chemottractant in mucin. Furthermore, it was observed that mucin can serve as a sole substrate for growth of *C. jejuni* (15), with studies at 42°C in PBS plus 1.0% mucin revealing an increase from 6 x 10^4 *C. jejuni* per ml at zero time to 3.3 x 10^5 and 2.4 x 10^5 *C. jejuni* per ml at 6 and 20 h, respectively (M. B. Hugdahl, J. T. Beery, and M. P. Doyle, unpublished data). Mucin served as a source of carbon and energy for growth, whereas in cultures containing PBS with or without other identified chemotactants of *C. jejuni*, the organism died off to undetectable levels (<10 CFU/ml) within 20 h at 42°C (15). Although only presumptive qualitative results could be obtained by staining cecal and cloacal crypt mucus of inoculated and control chicks with mucoprotein-glycoprotein-specific stains, it appeared that *C. jejuni* metabolizes mucus in vivo.

We propose that *C. jejuni* colonization of chicks occurs primarily in the lower gastrointestinal tract where the organ-
ism localizes principally in cecal and cloacal crypts. We further hypothesize that the chemoattraction of \textit{C. jejuni} to mucin attracts the organism to mucus, in which it moves by its highly active flagellum to mucus-filled crypts where the organism establishes itself. Within the crypts, \textit{C. jejuni} probably grows by using mucin as a substrate. The organism is likely to remain established in the crypt because of its attraction to and metabolism of mucin. \textit{C. jejuni} does not appear to attach to the crypt; adherence to the crypt is likely not important for colonization of the ceca and cloaca.

ACKNOWLEDGMENTS

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LITERATURE CITED


FIG. 3. High-power electron micrograph of proximal cecum of an 8-day-old chick inoculated with \textit{C. jejuni}. (A) Luminal-dwelling (nonadherent) bacteria are positively identified as \textit{C. jejuni} by immunoperoxidase staining in which the cell wall of \textit{C. jejuni} is dark staining, granular, and fuzzy coated. (B) Bacteria (subsequently confirmed as \textit{C. jejuni}) treated by the immunoperoxidase staining procedure except without the primary \textit{C. jejuni}-specific antibody (negative control); the bacteria lack the dark-staining, granular appearance of the cell wall. Also apparent is the lack of bacterial adherence to crypt microvilli. Peroxidase, osmium, uranyl acetate, lead citrate staining; Durcupan-embedded thin section. Bar, 1 μm.


