Host Attachment, Invasion, and Stimulation of Proinflammatory Cytokines by Campylobacter concisus and Other Non–Campylobacter jejuni Campylobacter Species

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Background. Campylobacter concisus and other non–Campylobacter jejuni Campylobacter species have been implicated in the initiation of gastrointestinal diseases. In the present study, we investigated the interaction between these bacteria and the human intestinal epithelium and immune cells.

Methods. The ability of C. concisus, Campylobacter showae, Campylobacter hominis, and Bacteroides ureolyticus to invade epithelial cells was examined using scanning electron microscopy and gentamicin protection assays. Proinflammatory cytokines generated by epithelial and immune cells in response to these bacteria were determined by enzyme-linked immunosorbent assay. Ussing Chamber, immunofluorescent stain, and Western blot were used to further elucidate the impact of C. concisus on intestinal barrier integrity and functions.

Results. Attachment of non–C. jejuni Campylobacter species to Caco-2 or HT-29 cells was mediated by flagellum-dependent and/or -independent processes. C. concisus was able to invade Caco-2 cells, generate a membrane-ruffling effect on the epithelial surface on entry, and damage epithelial barrier functions by preferential attachment to the cell-cell junctions. Proinflammatory cytokine profiles exhibited by epithelial cells, monocytes, and macrophages in response to C. concisus and other non–C. jejuni Campylobacter species were species and strain specific.

Conclusions. These findings demonstrate that C. concisus and other non–C. jejuni Campylobacter species may play a role in initiating gastrointestinal diseases.

Members of the Campylobacter genus are gram-negative spiral, curved, or rod-shaped organisms that usually inhabit the gastrointestinal tract of humans and animals. In recent years, an increasing number of Campylobacter species other than Campylobacter jejuni have been recognized as important human and veterinary pathogens; many of these have been implicated as causative agents of gastroenteritis of unknown etiology [1, 2]. Campylobacter species other than C. jejuni and Campylobacter coli are relatively fastidious and require specific hydrogen-enriched microaerobic environments for growth. Such conditions are not frequently used in routine clinical laboratories, which has led to their infrequent isolation and, thus, underestimation of their pathogenic potential [3, 4].

Very recently, we found an association between Campylobacter concisus and a number of other relatively unrecognized Campylobacter species and children with newly diagnosed Crohn’s disease [5, 6], a chronic and debilitating disease of the gastrointestinal tract with an unknown etiology. After our initial findings, Lastovica [7] reported the isolation of C. concisus from children and adults with Crohn’s disease. Early molecular studies...
have shown that *C. concisus* and other non- *C. jejuni* Campylobacter species may harbor a toxin similar to the cytolethal distending toxin [8, 9]. Furthermore, *C. concisus* isolates have been shown to secrete hemolysins, which affect human and animal red blood cells [10]. Specific strains of *C. concisus* may colonize the ileum, jejunum, and liver of wild-type BALB/cA mice and induce diarrhea, weight loss, and occasional liver microabscess formation [11]. The mechanism of interaction between *C. concisus* and human intestinal epithelial cells remains unknown.

Currently, none of the frequently detected Campylobacter species in children with newly diagnosed Crohn’s disease is well-characterized, including *Campylobacter showae*, *Campylobacter hominis*, and *Bacteroides ureolyticus*, which is a misclassified *Campylobacter* species [12, 13]. Although the pathogenesis of *C. showae* is completely elusive, a case report documented isolation of *C. hominis* in a blood sample from a septicemic patient [14]. *B. ureolyticus* may cause a loss of ciliary activity and sloughing of epithelial cells lining human fallopian tubes and bovine oviduct organ cultures [15]. Clearly, our knowledge of the repertoire of pathogenic mechanisms used by these non- *C. jejuni* Campylobacter species to cause disease in humans is limited. In the present study, we investigated the interaction between *C. concisus* and other non- *C. jejuni* Campylobacter species and human intestinal epithelial cells in vitro, including their ability to attach, invade, compromise intestinal barrier integrity, and induce proinflammatory cytokine production.

**MATERIALS AND METHODS**

**Bacterial species and strains.** The following bacterial species and strains were used: *C. concisus* UNSWCD, *C. concisus* ATCC51561, *C. concisus* ATCC51562, *C. concisus* UNSWCS, *C. showae* UNSWCD, *C. hominis* UNSWCD, *B. ureolyticus* UNSWCD, *Salmonella* Typhimurium LT2, and *Escherichia coli* K-12 (UNSW Culture Collection).

**Scanning electron microscopy.** Human intestinal epithelial cell lines Caco-2 or HT-29 were grown on glass cover slips in 24-well plates at a concentration of 5 × 10³ cells per well for 48 h. Cells were then infected with bacteria (multiplicity of infection [MOI], 200) for 6 h, washed with phosphate-buffered saline, and fixed overnight with 2% glutaraldehyde and 2.5% paraformaldehyde in 0.1 mol/L phosphate buffer (pH, 7.2). After dehydration in ethanol, monolayers were subjected to critical point drying with use of a critical point dryer (CPD 030; BAL-TEC), mounted on carbon tabs, and gold-coated in an Emitech K-550X Sputter Coater (Emitech). Samples were visualized on a Hitachi S3400 Scanning Electron Microscope (Hitachi).

**Gentamicin protection assays.** Caco-2 monolayers were infected with bacteria (MOI, 200) for 6 h, washed, and treated with 200 μg/mL of gentamicin (Gibco) for 1 h. Monolayers were lysed in 1% Triton X-100 and plated on suitable media. Colony-forming units were counted on plates after incubation for 2 days at 37°C. Time course experiments (30 min to 6 h) were also conducted for *C. concisus* UNSWCD. To determine the effect of pre-existing inflammation or cytoskeletal inhibitors on bacterial invasion, Caco-2 cells grown as described were treated with 40 ng/mL of tumor necrosis factor (TNF)–α or interferon (IFN)–γ, 2 μmol/L of cytochalasin D, or 10 μmol/L of colchicine (Sigma) for 1 h before the assay.

**Transepithelial electrical resistance (TER) and the Ussing Chamber.** Caco-2 cells were grown on 0.4-μm snap-well polycarbonate membrane supports (Corning). When TER reached a stable plateau, the monolayers were apically infected with *C. concisus* UNSWCD or *E. coli* K-12 (MOI, 200) and incubated for 6 h. Monolayers were washed with phosphate-buffered saline, and TER was measured using an EVOM and END0HM-24SNAP. Short-circuit current was measured using the Ussing Chamber (World Precision Instruments). To measure macromolecular permeability across the monolayer, horseradish peroxidase (HRP; Sigma) was added to the apical chamber to a final concentration of 1 × 10⁻³ mol/L at the baseline time. After 1 h, 500-μL aliquots were collected from the basal compartments, and HRP activity was estimated using an assay described elsewhere [16].

**Immunofluorescent staining and confocal laser scanning microscopy.** Caco-2 cells were grown on membrane supports and infected with *C. concisus* UNSWCD, as described above. Tight junctions were labeled with 10 μg/mL of anti–ZO-1 mouse monoclonal antibodies (Invitrogen), and *C. concisus* UNSWCD was labeled with rabbit anti–*C. concisus* serum (1:40) for 1.5 h. Goat anti-mouse secondary antibodies (Invitrogen) conjugated with Alexa Fluor 594 (5 μg/mL) and anti-rabbit secondary antibodies Alexa Fluor 488 (5 μg/mL) were then added and visualized using an inverted Olympus Fluoview FV1000 Confocal Laser Scanning Microscope (Olympus).

**One-dimensional polyacrylamide gel electrophoresis and Western blotting.** Proteins were extracted using the method of Wroblewski et al [17], separated on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels, and transferred to methanol-treated polyvinylidene difluoride membranes with use of the Trans-blot cell transfer system (Bio-Rad). Membranes were probed in accordance with the Immun-Star Western C Kit protocol (Bio-Rad). Membranes were immunolabeled with mouse monoclonal antibodies against ZO-1 (1:50), occludin (1:200), or β-actin (1:1000) (Santa Cruz). Goat anti-mouse IgG antibodies coupled to HRP (1:2000; Bio-Rad) were used as a secondary antibody.

**Enzyme-linked immunosorbent assay.** The level of interleukin (IL)–8 secreted in the supernatant by HT-29 cells was measured using the Quantikine Human CXCL8/IL-8 Kit ac-
Pathogenesis of *Campylobacter* Species

**RESULTS**

**Attachment and invasion by *C. concisus* and other non-*C. jejuni*** Campylobacter species. Scanning electron microscopy revealed that the *C. concisus* UNSWCD polar flagellum mediated initial contact with host cells via a flagellum-microvilli interaction. The flagellum was observed to attach to ≥1 microvillus (Figure 1A and 1C) and to wrap around a microvillus (Figure 1B), allowing the bacterium to anchor to the intestinal epithelial cell. After initial attachment, *C. concisus* UNSWCD penetrated the host cell membrane from the nonflagellated end, inducing a membrane ruffling–like effect on the host cell membrane (Figure 1C). Invasion appeared to proceed from the nonflagellated end, inducing a protrusion on the surface of the host cell membrane (Figure 1D).

As observed in Caco-2 cells, *C. concisus* UNSWCD also appeared to use its polar flagellum to facilitate attachment to HT-29 cells (Figure 2F). In addition, the nonflagellated end of *C. concisus* UNSWCD mediated attachment by a mechanism resembling a sticky end to attract nearby microvilli (Figure 2F). *C. concisus* UNSWCD appeared to preferentially attach to areas resembling intercellular junctional spaces (Figure 2A and 2B). This spatial distribution was augmented in the presence of TNF-α (Figure 2C–2E).

Investigation of *C. concisus* ATCC 51562, *C. showae*, *C. hominis*, and *B. ureolyticus* attachment to Caco-2 or HT-29 intestinal epithelial cells showed that *C. concisus* ATCC 51562 and *C. showae* appear to use their polar flagella to facilitate attachment to host cells. In contrast, *C. hominis* and *B. ureolyticus* appear to rely on other mechanisms to mediate initial contact with host cells.
Figure 2. Preferential attachment of *Campylobacter concisus* UNSWCD to intercellular junctional spaces of HT-29 cells. A and B, *C. concisus* UNSWCD preferentially attached to areas resembling the intercellular junctional space. F. The process of attachment was mediated by the polar flagellum (arrows). Bacterial attachment was also mediated by the aflagellate end (triangles). C–E, When HT-29 cells were pretreated with tumor necrosis factor–α before infection, a higher density of bacterial attachment was observed, especially in areas resembling intercellular junctional spaces, but also at apical surfaces (C, triangles).

*showae* used flagellum-mediated attachment to microvilli. Similar to *C. concisus* UNSWCD, *C. concisus* ATCC 51562 (Figure 3D) and *C. showae* (Figure 3B) used their polar flagellum to adhere intimately to the microvilli. The nonflagellated end of *C. concisus* ATCC 51562 and *C. showae* also facilitated attachment by attracting neighboring microvilli on the bacterial surface with use of a sticky end mechanism (Figure 3A, 3B, and 3D). In Caco-2 and HT-29 cell lines, minimal attachment was observed for the aflagellate *B. ureolyticus* and *C. hominis*. Where attachment was observed, they also appeared to use a flagellum-independent mechanism of attachment (Figure 3C, 3E, and 3F).

**Quantification of bacterial invasion levels.** The ability of *C. concisus, C. showae, C. hominis*, and *B. ureolyticus* to invade Caco-2 cells at an MOI of 200 was further evaluated and quan-
Attachment to HT-29 or Caco-2 cells by non--Campylobacter jejuni Campylobacter species, mediated by processes dependent or independent of a polar flagellum. Similar to Campylobacter concisus UNSWCD strains, Campylobacter showae (B) and C. concisus ATCC 51562 (D) used their polar flagellum to adhere intimately to the microvilli (triangles). A, B, and D. The nonflagellated end of C. showae (A and B) and C. concisus ATCC 51562 (D) also facilitated in the attachment process by attracting neighboring microvilli to the bacterial surface (sticky end mechanism; arrows). Aflagellate Campylobacter species also used a flagellum-independent mechanism of attachment to HT-29 cells (C, Bacteroides ureolyticus; arrow indicates sticky end) or Caco-2 cells (E, B. ureolyticus; F, Campylobacter hominis).

Identified using gentamicin protection assays. Of the Campylobacter species examined, C. concisus UNSWCD was the most efficient in invading Caco-2 cells. The mean percentage (± standard error of the mean [SEM]) of invasion by C. concisus UNSWCD was 0.14% ± 0.04%. Less than 0.01% viable intracellular C. concisus ATCC 51562 and UNSWCS was recovered from Caco-2 cells. The level of invasion observed in C. concisus UNSWCD was 46 and 201 times higher than that of C. concisus UNSWCS and C. concisus ATCC 51562, respectively. C. concisus ATCC 51561, C. showae, C. hominis, and B. ureolyticus did not invade Caco-2 cells, consistent with our observations obtained using scanning electron microscopy. The positive control S. Typhi-
Figure 4. Increased barrier permeability but not ion transportation in Caco-2 cells after infection with Campylobacter concisus UNSWCD. After infection with C. concisus UNSWCD, the transepithelial electrical resistance (TER) of the Caco-2 monolayer decreased significantly (A), whereas no change in TER was recorded in Caco-2 monolayers infected with Escherichia coli K-12 (B). Movement of horseradish peroxidase (HRP) from the apical to basal compartment was measured (C), and a significantly higher flow-through was detected in Caco-2 monolayers infected with C. concisus UNSWCD, compared with E. coli K-12 (P = .029). No statistically significant difference was observed in the short-circuit current of Caco-2 monolayers infected with C. concisus UNSWCD (D), compared with E. coli K-12 (E) over 75 min after 6 h of infection (P = .065).

murium, as expected, exhibited the highest overall levels of invasion, with a mean level (±SEM) of 1.92% ± 0.31%. The number of viable extracellular bacteria after gentamicin treatment was nil or <1% of total recoverable bacteria.

C. concisus infection is dependent on time, pre-existing inflammation, and host cytoskeleton. Because C. concisus UNSWCD showed the greatest capacity to invade human epithelial cells, a time course experiment was conducted to determine whether the process of invasion was dependent on time and host cytoskeleton. The maximum level of invasion was observed after 6 h of incubation and was therefore expressed as 100%. The mean percentage (±SEM) of C. concisus UNSWCD invasion after 30 min, 2 h, and 4 h of incubation was 6.5% ± 3.1%, 34.3% ± 9.5%, and 3.7% ± 2.3%, respectively.

Investigation of the potential impact of gut inflammation driven by cytokines, such as TNF-α [18] and IFN-γ [19], that was observed in patients with Crohn’s disease on C. concisus UNSWCD invasion showed that the ability of C. concisus UNSWCD to invade Caco-2 cells treated with TNF-α was significantly increased by a mean percentage (±SEM) of 62% ± 7.6%, compared with untreated Caco-2 cells (P = .015). Similarly, a significantly elevated level of invasion was observed in Caco-2 cells treated with IFN-γ, compared with untreated Caco-2 cells (mean increase ± SEM, 36% ± 5.9%; P = .027). In addition, C. concisus UNSWCD invasion was significantly attenuated in the presence of colchicine (inhibits microtubule polymerization; P = .006), decreasing from 100% to a mean percentage (±SEM) of 15.7% ± 6.6%. Similarly, the level of invasion significantly decreased when Caco-2 cells were treated with cytochalasin D (inhibits microfilaments; P = .001), from 100% to a mean percentage (±SEM) of 28.2% ± 2.4%.

C. concisus–induced movement of tight junction proteins significantly compromising TER and membrane permeability. The effect of C. concisus UNSWCD on intestinal barrier function was further investigated by measuring TER, membrane
Figure 5. Redistribution of ZO-1 in Caco-2 cells infected with *Campylobacter concisus* UNSWCD. A Caco-2 monolayer in the absence of bacteria showing an intact ZO-1 boundary (red) confined to the intercellular junction (A). After infection with *C. concisus* UNSWCD (green), bacterial attachment and degradation of ZO-1 was observed after 6 h (B) or 48 h of infection (C). B, Disruption and movement of ZO-1 from the cell-cell boundary, as a result of infection with *C. concisus* UNSWCD for 6 h, is indicated by arrows. C, Loss of ZO-1 was more pronounced at 48 h. Views of cross-sectional images revealed the presence of *C. concisus* UNSWCD below ZO-1 proteins, indicating the occurrence of bacterial translocation across the cell-cell junction after 6 h (B) or 48 h of infection (C). D, Magnified view of a ZO-1 boundary, showing preferential attachment of *C. concisus* UNSWCD to the cell-cell junction and induction of ZO-1 internalization along the side with extensive bacterial attachment.

permeability, and ion transportation of infected Caco-2 monolayers. The TER value of Caco-2 cells stabilized and reached a plateau after 30 days of cultivation on cell culture membrane supports. After a 6-h infection of stabilized Caco-2 monolayers with *C. concisus* UNSWCD, the TER was significantly reduced (mean TER ± SEM, 52.0 ± 4.1 ohm/cm²), compared with before infection (mean TER ± SEM, 249.5 ± 5.6 ohm/cm²; *P* < .001) (Figure 4A). In contrast, the TER of Caco-2 monolayers was not affected after infection with *E. coli* K-12 (mean TER ± SEM before infection, 211.0 ± 11.5 ohm/cm²; mean TER ± SEM after infection, 211.0 ± 10.9 ohm/cm²; *P* > .99) (Figure 4B). In addition, increased membrane permeability as a result of infection with *C. concisus* UNSWCD was detected using HRP translocation in the Ussing Chamber (Figure 4C). Caco-2 monolayers infected with *C. concisus* UNSWCD showed significantly elevated levels of HRP in the basolateral compartment (mean level ± SEM, 877.3 ± 198 nmol/L), compared with monolayers infected with the *E. coli* K-12 negative control (mean level ± SEM, 93.4 ± 29.7 nmol/L; *P* = .029). After a 6-h infection with *C. concisus* UNSWCD, the short-circuit cur-
Figure 6. Movement of tight junction protein ZO-1 and occludin from host membrane to the cytosol of Caco-2 cells induced by Campylobacter concisus UNSWCD. A, B, and D. The level of ZO-1 or occludin significantly decreased in the membrane (insoluble; IS) fraction, and ZO-1 also increased in the cytosolic (soluble; S) fraction. A, C, and E. Total content of ZO-1 or occludin remained unchanged after infection with C. concisus UNSWCD for 48 h. β-actin was used as a loading control. Data from 3 independent experiments. *; **. 

![Figure 6](image-url)
Figure 7. A, Levels of interleukin (IL–8) produced by the human intestinal epithelial cell line HT-29 after infection with viable or heat-killed non–Campylobacter jejuni Campylobacter species. B, Levels of IL-1β, IL-8, and tumor necrosis factor (TNF)–α produced in the THP-1 human monocytic cell line and primary human macrophage. Data from 3 independent experiments (± standard error of the mean), with each experiment performed in duplicate. MOI, multiplicity of infection. *P < .05; **P < .01.
and B. ureolyticus were comparable to those of their viable counterparts but were not significantly elevated, compared with that induced by heat-killed E. coli K-12.

C. concisus and other non–C. jejuni Campylobacter species stimulated the production of proinflammatory cytokines in a monocytic cell line and primary macrophages. After entry into the submucosa and bloodstream through a compromised epithelial barrier, pathogens encounter monocytes and macrophages. Examination of the THP-1 monocytic cell response to bacterial stimulation revealed that C. concisus UNSWCD, C. concisus ATCC 51561, and C. hominis induced significantly elevated levels of IL-8 and TNF-α, but not IL-1β, compared with unstimulated controls (Figure 7B). C. hominis induced the highest level of IL-8 in all bacteria tested. In contrast, C. concisus ATCC 51562, C. showae, and B. ureolyticus stimulated relatively low levels of IL-8 and TNF-α in THP-1 monocytes, suggesting that the ability of Campylobacter species to stimulate these proinflammatory cytokines is both strain and species dependent. All Campylobacter species tested induced relatively low IL-1β levels. Only S. Typhimurium stimulated a significantly higher level of IL-1β in THP-1 monocytes, compared with unstimulated monocytes (P < .01).

Primary human macrophages infected with C. concisus UNSWCD, C. concisus ATCC 51561, C. concisus ATCC 51562, and C. hominis produced a significantly higher level of TNF-α, compared with the unstimulated control, whereas C. showae, B. ureolyticus, and S. Typhimurium did not (Figure 7B). Only S. Typhimurium induced a significantly elevated level of IL-1β, compared with the unstimulated control. All other Campylobacter species induced relatively low levels of IL-1β. Although all Campylobacter species and S. Typhimurium induced increased levels of IL-8 in primary human macrophages, the levels observed were not significantly higher than those in the unstimulated control.

**DISCUSSION**

Although pathogenic mechanisms of C. jejuni are well documented, there have been limited studies investigating the pathogenesis of non–C. jejuni Campylobacter species. Non–C. jejuni Campylobacter species are important human and veterinary pathogens, and a number of these, including C. concisus, are currently considered to be emerging pathogens of the human intestinal tract. To investigate the potential of these organisms to initiate disease, we examined the pathogen-host relationships with use of human epithelial cell lines and immune cells. Our investigations using scanning electron microscopy showed that the polar flagella of both C. concisus and C. showae were involved in host cell attachment. The flagellum from these bacteria was observed to attach to the tips of microvilli, a process that may be required for colonization and, for C. concisus UNSWCD, penetration in the host cell. In C. jejuni, attachment is also necessary for facilitating the injection of Campylobacter invasion antigens into host cells [20]. Of interest, C. jejuni mutants defective in the major flagellin subunit K2–32 have reduced attachment and invasion capacity [21], suggesting the role of flagella in determining the invasiveness of Campylobacter species. After flagellum-mediated attachment, C. concisus UNSWCD initiated a membrane-ruffling effect on the host cell membrane in a manner not very dissimilar to that of Salmonella Typhi invasion [22].

In addition, we found visual evidence that a nonflagellate sticky end mechanism of attachment appears to exist for all Campylobacter species examined. The sticky end of these bacteria appears to attract nearby microvilli to the bacterial cell surface, possibly mediated by the presence of bacterial surface adhesins. This mechanism of attachment represents another strategy by which members of the Campylobacter genus adhere to intestinal epithelial cells, which is particularly advantageous for the aflagellate B. ureolyticus and C. hominis. Although B. ureolyticus, C. hominis, and C. showae had the ability to attach to intestinal epithelial cells, they did not appear to be able to invade. A strain of C. concisus isolated from a child with Crohn’s disease had the greatest ability to invade Caco-2 intestinal epithelial cells, compared with the 3 other non–Crohn’s disease strains. Comparison of the level of invasion by C. concisus UNSWCD with those of previously documented Campylobacter species revealed striking similarities [23, 24] and suggests that only a small population of Campylobacter may invade or survive in intestinal epithelial cells.

In addition to a transcellular route of infection through host invasion, we showed that C. concisus UNSWCD preferentially attached to intercellular junctional spaces and possibly translocated across the epithelium through a paracellular route. Such spatial distribution was concomitantly associated with a striking decrease in TER, an increase in HRP flux, and a loss of membrane-associated ZO-1 and occludin in Caco-2 monolayers after C. concisus UNSWCD infection. The observed preferential attachment may allow rapid disruption of tight junctions, either by direct C. concisus–host contact or by release of secretory proteins directly to the target sites, resulting in damage to the structural integrity and function of intestinal epithelial cells. Although the precise signal required is currently unknown, it is possible that internalization of ZO-1 and occludin from the cell membrane to the cytosol induced by C. concisus UNSWCD is attributable to a zonula occludens toxin, which has been identified in the complete genome of C. concisus strain 13826 [25]. Although the mechanism of zonula occludens toxin in C. concisus remains to be deciphered, studies in Vibrio cholerae have shown that zonula occludens toxin significantly increases tissue permeability, initiates actin polymerization, and increases intestinal secretion [26, 27].

In addition to attachment and invasion, our results showed
that C. concisus strains, irrespective of their invasive abilities, stimulated significantly elevated levels of IL-8, compared with E. coli K-12, in HT-29 cells. Furthermore, direct contact with viable C. concisus and other non-C. jejuni Campylobacter species was not required for IL-8 production. C. concisus also exhibited a strain dependency in the ability to stimulate IL-8 and TNF-α in monocyctic THP-1 cells. C. hominis stimulated the highest levels of IL-8 and TNF-α in both monocytes and macrophages of all bacteria tested, including S. Typhimurium. Whether the potent stimulatory ability of C. hominis contributed to a reported fatal septicemia [14], in which C. hominis was isolated from the blood of a patient, is intriguing.

In conclusion, we revealed with use of an in vitro model that non–C. jejuni Campylobacter species, in particular C. concisus, have the potential to invade and modulate barrier permeability and cytokine production, suggesting that they are unlikely to be commensals of the intestinal tract and have the potential to cause disease. Such findings provide a novel insight into the role of non–C. jejuni Campylobacter species in inflammatory bowel diseases and other infectious gastrointestinal diseases with unknown etiology. Examination of additional clinical isolates is required to ascertain whether the virulence factors displayed by C. concisus and the other non–C. jejuni Campylobacter species are reproducible.

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References