Differences in virulence attributes between cytolethal distending toxin positive and negative *Campylobacter jejuni* strains

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Campylobacter jejuni is a common gastrointestinal bacterial pathogen. Although cytolethal distending toxin (CDT) is proposed to be an important virulence determinant of this pathogen, how CDT⁺ and CDT[−] strains differ in their biological properties remains largely unknown. The virulence properties of CDT⁺ and CDT[−] strains were studied on HeLa cells and in the suckling mouse model. Presence of the *cdtB* gene in *Campylobacter* species was determined by PCR. Five each of CDT⁺ and CDT[−] *C. jejuni* strains were subjected to adherence, invasion and cytotoxicity assay on the HeLa cell line. Bacterial culture supernatants with and without CDT activity were inoculated intragastrically into 2-day-old suckling mice. The mice were sacrificed within 48 h. Histopathological examination of stomach, jejunum, ileum and colon was performed by haematoxylin/eosin staining. *cdtB* was detected in 88% and 14% of *C. jejuni* and *Campylobacter coli* strains, respectively. CDT⁺ *C. jejuni* strains adhered to and invaded HeLa cells in significantly higher numbers than CDT[−] strains [CDT⁺ vs CDT[−], adherence $2.7 \times 10^4 \pm 3.5 \times 10^4$ vs $2.7 \times 10^2 \pm 1.9 \times 10^2$; invasion $1.0 \times 10^3 \pm 1.3 \times 10^3$ vs $1.4 \times 10^1 \pm 3.1 \times 10^1$; $P < 0.01$]. Culture supernatants of all CDT⁺ strains demonstrated CDT activity on HeLa cells. Mice inoculated with supernatant containing CDT activity had moderate to severe pathology in different parts of their gastrointestinal tract, with the colon being the major target. Mice inoculated with supernatant lacking CDT activity showed no significant pathology in the gastrointestinal tract. The results demonstrate that CDT⁺ *C. jejuni* strains adhere to and invade epithelial cells more efficiently than CDT[−] strains. CDT is responsible for intestinal pathology and the colon is the major target.

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INTRODUCTION

Campylobacter is a major cause of food-borne bacterial enteritis worldwide. It is estimated that the true *Campylobacter* infection rate in the USA and UK is as high as 1% of the population per year (Linton *et al.*, 1997). In the developing countries the infection rate varies from 5 to 20% in children with diarrhoeal illness (Oberhelman & Taylor, 2000). The clinical spectrum ranges from a mild self-limiting, non-inflammatory diarrhoea to severe inflammatory bloody diarrhoea with abdominal pain and fever (Ketley, 1997; Ismael *et al.*, 2005).

The pathogenesis of *Campylobacter* infection is multifactorial and complex. Various virulence factors such as adherence, invasive capabilities and toxin production have

been implicated (Konkel *et al.*, 2001). Adherence of bacteria to the epithelial cell surface is probably an important determinant for colonization and may increase the local concentration of secreted bacterial products; however, invasion is accompanied by pronounced cytopathic effects and is thought to be a primary mechanism of damage to the colonic mucosa, leading to inflammation (Russell *et al.*, 1993; Wooldridge & Ketley, 1997). *Campylobacter* strains invade the gastrointestinal tract, and damaged epithelial cells are exfoliated into the lumen (Russell *et al.*, 1993). Invaded cells become swollen and rounded, indicating changes in ion transport regulators, probably due to the production of cytotoxins (Friis *et al.*, 2005). Toxins have been considered important factors for the pathogenesis of *Campylobacter* infection. The best-characterized of the toxins attributed to *Campylobacter* spp. is cytolethal distending toxin (CDT). The *C. jejuni* *cdt* operon consists of three adjacent genes, *cdtA*, *cdtB* and *cdtC*, that encode proteins with predicted molecular masses of 27, 29 and 20 kDa, respectively.

Abbreviations: CDT, cytolethal distending toxin; CDT[−], CDT-negative; CDT⁺, CDT-positive; FCS, fetal calf serum.

The GenBank/EMBL/DDBJ accession number for the *cdt** primers is DQ882648.

CdtB is proposed to be the enzymically active subunit of the holotoxin (Lara-Tejero & Galan, 2001; Smith & Bayles, 2006). Recent studies have shown that the *cdtB* gene is commonly present in *Campylobacter jejuni* rather than *Campylobacter coli* (Bang *et al.*, 2001; Eyigor *et al.*, 1999). CDT belongs to a family of bacterial protein toxins with the ability to block the cell cycle process, with resulting cell cycle arrest and cell death (De Rycke & Oswald, 2001). However, differences in the adherence and invasion capabilities of CDT-positive (CDT⁺) and negative (CDT⁻) strains of *C. jejuni* have not been studied. The strains of *Escherichia coli*, *Shigella* spp. and *Campylobacter* spp. that show CDT activity are associated with diarrhoeal disease (Bouzari *et al.*, 1992; Johnson & Lior, 1987, 1988). Several *in vitro* and *in vivo* models have been described to study the role of CDT in the pathogenesis of *E. coli* and *Shigella* infection (Johnson & Lior, 1987; Okuda *et al.*, 1997).

Although CDT production is more common in *C. jejuni* strains isolated from patients with inflammatory diarrhoea, how CDT⁺ and CDT⁻ strains differ in their virulence properties is not yet known. The present study was planned to determine the difference between CDT⁺ and CDT⁻ *C. jejuni* strains in their adherence and invasive properties on the HeLa cell line and biological activities in the suckling mouse model.

METHODS

Bacterial strains. A total of 49 *Campylobacter* strains (41 *C. jejuni*, 7 *C. coli* and 1 *C. lari*) isolated from the rural community of Lucknow district, India, were tested for the presence of the *cdtB* gene. Details of the species identification of these isolates have been described (Jain *et al.*, 2005). Ten *C. jejuni* strains (five CDT⁻ and five randomly selected CDT⁺ *C. jejuni* strains) were subjected to adherence and invasion assay on HeLa cells. Culture supernatants from these *C. jejuni* strains were tested for CDT activity on HeLa cells.

Cell line. The HeLa (human cervical adenocarcinoma) cell line was used for the adherence, invasion and cytotoxin assay of *C. jejuni* strains. The cell line was maintained in Eagle's minimum essential medium (MEM) with 10% fetal calf serum (FCS).

Detection of *cdtB* in *Campylobacter* species by PCR. Genomic DNA was extracted from *Campylobacter* strains by the alkaline lysis method (Sambrook *et al.*, 1989). The DNA was subjected to PCR using specific primers for the *cdtB* gene. *cdtB** gene primers, developed in our laboratory using Biosoftware (Primer Premier, PREMIER Biosoft International) were as follows: upstream 5'-GCGTTGATGTAGGAGCTAATCGTG-3' and downstream 5'-GGTTGATCGCGTTGAGTTCGTT-3'. The primer sequences have been submitted to GenBank (accession no. DQ882648). Another set of primers (upstream 5'-GTAAAAATCCCCTGCTATCAACCA-3' and the downstream 5'-GTTGGCACTTGGAATTTGCAAGGC-3') for the *cdtB* gene reported by Bang *et al.* (2001) was used to confirm the results of our laboratory-developed *cdtB** gene primers.

All PCR amplifications were performed in a 50 µl volume containing 10 × assay buffer, 200 µM each dATP, dCTP, dGTP, dTTP, 0.1 µM each primer, 1.5 units *Taq* DNA polymerase (Bangalore Genei, India) for 30 cycles with the following programs. *cdtB** primers: 1 min at 94 °C, 1 min at 60 °C, 1 min at 74 °C followed by extension of

10 min at 74 °C; *cdtB* primers (Bang *et al.*, 2001): 1 min at 94 °C, 2 min at 42 °C, 3 min at 72 °C and extension of 10 min at 72 °C.

Adherence and invasion assay. Tissue culture plates (Nunc, 24-well) were seeded with 5×10^4 cells ml⁻¹. The plates were incubated at 37 °C in a humidified 5% CO₂ incubator (Sanyo) until semi-confluent monolayers were obtained. Prior to the experiment, the cells were washed twice with phosphate-buffered saline (PBS) and incubated with MEM containing 2% FCS.

Suspensions of CDT⁺ and CDT⁻ *C. jejuni* strains were prepared in PBS (pH 7.0) from cultures grown on charcoal cefoperazone deoxycholate agar (CCDA) under microaerophilic conditions at 37 °C for 48 h. The suspension was centrifuged at 10 000 g for 10 min at 4 °C. The bacterial pellet was resuspended in MEM with 2% FCS, and the inoculum was adjusted to 10^7 – 10^8 bacteria ml⁻¹ by measuring the OD₆₀₀. The adherence and invasion assays were done by methods described earlier (Konkel *et al.*, 1992; Prasad *et al.*, 1996). The inoculum (0.5 ml) was added to the HeLa cell monolayer in duplicate. The plates were incubated at 37 °C in a 5% CO₂ incubator for 3 h. The monolayers were washed three times with MEM containing 2% FCS. In one of the duplicate wells, gentamicin (250 µg ml⁻¹) was added and plates were further incubated for 3 h. *C. jejuni* being sensitive to gentamicin, the antibiotic killed the bacteria that adhered to the surface of the tissue culture, while the bacteria that had already invaded and internalized remained unaffected. In the second well, medium without antibiotic was added to determine the number of bacteria that had adhered to and invaded the cell lines. The monolayers were lysed using 0.01% Triton X-100. The lysed monolayer suspensions were diluted (10^{-1} to 10^{-4}) in PBS, and 100 µl of each dilution was uniformly plated on CCDA. The number of viable bacteria was determined by counting the c.f.u. on the plates, multiplied by the dilution factor. Viable bacteria recovered from the first well (with gentamicin) and from the second well (without gentamicin) represented the intracellular (invasion) and the extra-cellular + intracellular (adherence + invasion) bacterial counts, respectively. The adherence was calculated by the formula [(c.f.u. ml⁻¹ from the second well at particular dilution) – (c.f.u. ml⁻¹ from the first well at the same dilution) × dilution factor].

Cytotoxicity assay on HeLa cells. Cell-free bacterial culture supernatants from all the strains were prepared according to the method described by Florin & Antillon (1992) with minor modifications. Briefly, each of five CDT⁺ and CDT⁻ strains was harvested from CCDA plates into MEM cell culture medium. The volume of medium used was adjusted so that the OD₆₀₀ of the bacterial suspension was 0.125 (2×10^8 c.f.u. ml⁻¹). The bacterial count measured by optical density was subsequently confirmed by colony counts using different dilutions on solid media. Bacterial strains suspended in MEM tissue culture medium were lysed by sonication (4 × 30 s bursts with 30 s intervals between each burst). Cell debris and unlysed bacteria were then removed by centrifugation at 10 000 r.p.m. for 20 min at 5 °C and filter (0.22 µm) sterilized. The culture supernatant was then dialysed 10-fold using polyethylene glycol 600 (SRL, India) and finally the concentrated supernatant was collected and stored at –20 °C until use.

HeLa cells were seeded into 24-well tissue culture plates (Nunc) at a density of 2×10^4 cells per well in 0.5 ml medium. Doubling dilutions of culture filtrates were prepared in MEM and 0.5 ml of each dilution was added to the HeLa cells and incubated for 3 days at 37 °C in an atmosphere of 5% CO₂. The cells were examined under an inverted microscope every 24 h up to 72 h for demonstration of morphological changes. CDT activity titre was defined as the reciprocal of the highest dilution that produced distension in >50% of the cells. Adherence, invasion and cytotoxicity experiments were done in triplicate and the mean results were considered for further analysis.

Suckling mice and sample inoculation. Suckling mice (2 days old) were obtained from the animal house, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow. Prior to the sample inoculation, mice were starved by separating them from their mother for 10 h at 23 °C. A total of 20 suckling mice were included in the study and divided into two groups of 10. Each group of mice was inoculated with culture supernatants of five CDT⁺ *C. jejuni* and five CDT⁻ *C. jejuni* strains, each strain in duplicate animals. Culture supernatant mixed with Evans blue dye (0.04 %, v/v) was administered into the stomach of each suckling mouse. The mice were observed for the development of diarrhoea and were sacrificed within 48 h.

Histopathology. Parts of the small and large intestines (stomach, jejunum, ileum and colon) were dissected from the suckling mice and fixed in 10 % formalin. Multiple representative areas from each anatomical region were processed for paraffin-embedded sections; 4 µm sections were stained with haematoxylin and eosin for morphological examination. Histopathological evaluation was done blinded to the inoculation subgroups. The extent of inflammation and mucosal damage was categorized as mild (+), moderate (++) or severe (+++).

Statistical analysis. All the statistical analyses were done with SPSS statistical software, version 12.0 (SPSS Inc.). Comparison of adherence and invasion assay among CDT⁺ and CDT⁻ *C. jejuni* strains was done using the Kruskal–Wallis test.

RESULTS

Detection of the *cdtB* gene in *Campylobacter* isolates by PCR

Among the 41 *C. jejuni*, 7 *C. coli* and 1 *C. lari* isolates, the *cdtB* gene was present in 36 *C. jejuni* and 1 *C. coli*; the lone *C. lari* strain was negative for *cdtB* (Table 1). Significantly higher proportions of *C. jejuni* strains contained *cdtB* as compared to *C. coli* (36/41 vs 1/7; $P < 0.001$). The results of both sets of primers (*cdtB*⁺ and *cdtB*) were identical.

Adherence and invasion assay

The CDT⁺ strains had significantly higher adherence and invasive capabilities to HeLa cells as compared to CDT⁻ strains. The details of adherence and invasion assays are shown in Table 2. CDT⁺ *C. jejuni* strains isolated from diarrhoeal patients adhered to HeLa cells with a range of 8.0×10^2 to 7.2×10^4 c.f.u. ml⁻¹ (mean $2.7 \times 10^4 \pm 3.5 \times 10^4$); all five CDT⁺ *C. jejuni* strains tested were found

Table 2. Adherence and invasion assay of CDT⁺ and CDT⁻ *C. jejuni* strains on the HeLa cell line

Strain	Adherence (c.f.u. ml ⁻¹)	Invasion (c.f.u. ml ⁻¹)
CDT⁺*		
837	6.2×10^4	3.1×10^3
556	7.2×10^4	1.5×10^3
728	1.6×10^3	1.2×10^2
726	2.5×10^3	1.4×10^2
314	8.0×10^2	2.0×10^2
Mean $\pm$ SD	$2.7 \times 10^4 \pm 3.5 \times 10^4$	$1.0 \times 10^3 \pm 1.3 \times 10^3$
CDT⁻*		
356	2.2×10^2	0
130	1.3×10^1	0
584	5.4×10^2	0
978	2.9×10^2	0
701	3.3×10^2	0.7×10^2
Mean $\pm$ SD	$2.7 \times 10^2 \pm 1.9 \times 10^2$	$1.4 \times 10^1 \pm 3.1 \times 10^1$

*CDT⁺ vs CDT⁻ strains, adherence ($P < 0.01$); invasion ($P < 0.01$).

to invade (internalize) HeLa cells. The CDT⁻ *C. jejuni* strains adhered to HeLa cells with a range of 1.3×10^1 to 5.4×10^2 c.f.u. ml⁻¹ (mean $2.7 \times 10^2 \pm 1.9 \times 10^2$). Among these strains, only one was found to be invasive.

Cytotoxicity assay on HeLa cells

CDT activity of *C. jejuni* culture supernatant was confirmed by distention and rounding of HeLa cells in all five CDT⁺ strains; CDT titres in these strains ranged from 1 in 32 to 1 in 64. None of the CDT⁻ isolates showed CDT activity.

Intragastric challenge of suckling mice with culture supernatant containing CDT activity

Watery diarrhoea within 24 h was observed in 6 of the 10 mice inoculated with supernatant containing CDT activity; the remaining 4 mice had no sign or symptoms of diarrhoea. Mice inoculated with supernatant lacking CDT activity did not show any sign of diarrhoea.

Histopathology

Histology was done on all 10 mice. A varying degree of inflammatory reaction was observed in different parts of the gastrointestinal tract. The changes were categorized as mild when a mild inflammatory infiltrate with no destructive changes was present. Moderate inflammation was defined as presence of moderate inflammatory exudates with partial necrosis of mucosa and luminal exudates. Tissues were categorized as having severe inflammation when the mucosa was almost completely denuded, with a prominent inflammatory infiltrate, necrosis and luminal exudates.

Table 1. Presence of the *cdtB* gene in various *Campylobacter* species

Species	<i>cdtB</i>	
	Positive	Negative
<i>C. jejuni</i> (n=41)	36 (87.8 %)*	5 (12.1 %)
<i>C. coli</i> (n=7)	1 (14.2 %)*	6 (85.7 %)
<i>C. lari</i> (n=1)	0	1

**C. jejuni* vs *C. coli* ($P < 0.001$).

A remarkable change in pathology was observed in gastrointestinal tissues of the mice challenged with supernatant containing CDT⁺ activity. Moderate to severe inflammation was observed in all parts of the gastrointestinal tract in six mice, while in the remaining four, the stomach was spared. The sections from stomach showed partial denudation of mucosa, and fibrinous exudates in the lumen, with prominent mixed inflammatory infiltrate extending to the submucosa (Fig. 1b). The jejunum showed disruption of the mucosal lining with intense panmural inflammatory infiltration (Fig. 1d). In the ileum, the mucosa was mostly denuded, broken glands were evident in the lumen and the lining was replaced by macrophages mixed with neutrophils and lymphocytes. Panmural inflammation was present (Fig. 1f). Sections from colon also showed denuded mucosa and mucosal inflammation with macrophages, neutrophils and lymphocytes; luminal necrotic exudate was also evident (Fig. 1h). All 10 mice inoculated with supernatant lacking CDT activity showed no significant pathology in the stomach, jejunum or ileum, with mild inflammation in the colon in four animals (Fig. 1a, c, e, g). The destructive damage and intense inflammatory response noted in intestinal tissues of mice challenged with supernatant containing CDT⁺ activity was not evident in any member of the CDT⁻ group. The pathological changes in tissues of the large and small intestine of mice are summarized in Table 3.

DISCUSSION

The presence of the *cdtB* gene among *Campylobacter* species isolated from a rural community of north India was investigated. Several publications have reported the frequency of the *cdtB* gene among *Campylobacter* isolates from different sources (Eyigor *et al.*, 1999; Florin & Antillon, 1992). In our study, 87.8% *C. jejuni* and 14.2% *C. coli* were found to be positive for *cdtB* by PCR. Similarly, Bang *et al.* (2001) reported *cdt* positivity in 90.4% of *C. jejuni* and 9.6% of *C. coli* strains. More recently, it has been reported from Louisiana that 100% of *C. jejuni* and *C. coli* isolates were positive for *cdtB* (Dassanayake *et al.*, 2005). In a study in Bahrain, among the 96 *C. jejuni* strains examined, 80 (83.0%) were *cdtB* positive and 16 (17.0%) were negative by PCR (Al-Mahmeed *et al.*, 2006).

The above findings suggest that the *cdtB* gene is present in the majority of *C. jejuni* strains. To prove the role of *cdtB* as virulence marker we assayed the adherence and invasion properties of *cdtB*⁺ and *cdtB*⁻ *C. jejuni* strains on HeLa cells. Interestingly, all *cdtB*⁺ strains adhered to (range 8.0×10^2 to 7.2×10^4 c.f.u. ml⁻¹, mean $2.7 \times 10^4 \pm 3.5 \times 10^4$) and invaded (range 1.2×10^2 to 3.1×10^3 c.f.u. ml⁻¹, mean $1.0 \times 10^3 \pm 1.3 \times 10^3$) HeLa cells, while the level of adherence (range 1.3×10^1 to 5.4×10^2 c.f.u. ml⁻¹, mean $2.7 \times 10^2 \pm 1.9 \times 10^2$) and invasion (range 100 to 0.7×10^2 c.f.u. ml⁻¹, mean $1.4 \times 10^1 \pm 3.1 \times 10^1$) by *cdtB*⁻ *C. jejuni* strains was significantly lower (only one CDT⁻ strain showed a low level of invasion). Biswas *et al.* (2006)

Fig. 1. Histopathological evaluation by haematoxylin and eosin staining of the gastrointestinal tract of mice challenged with culture supernatants of CDT⁺ and CDT⁻ *C. jejuni* strains. (a, b) Stomach: (a) CDT⁻, showing intact mucosa, minimal inflammation; (b) CDT⁺, showing partially denuded mucosa, fibrinous exudates in the lumen and mixed inflammatory infiltration. (c, d) Jejunum: (c) CDT⁻, showing normal jejunal morphology; (d) CDT⁺, showing partially denuded mucosa with intense inflammatory infiltrate. (e, f) Ileum: (e) CDT⁻, showing normal ileal morphology; (f) CDT⁺ showing denuded mucosa with broken glands in lumen and replaced by macrophages, mixed with neutrophils and lymphocytes. (g, h) Colon: (g) CDT⁻, showing intact mucosa with mild inflammation; (h) CDT⁺, showing denuded mucosa with a few remnant glands (arrow), inflammation with macrophages, neutrophils and lymphocytes and luminal necrotic exudates. Magnification: (g) $\times 125$; other panels $\times 525$.

recently reported that there was no difference in adhesion to HeLa cells of *cdt* mutants compared to wild-type, but that they did have reduced levels of invasion. The ability of mutants to colonize birds either directly or by horizontal transfer was unchanged. The discrepancy in adherence properties between the studies might be related to the bacterial strains, as in the present study strains deficient in *cdtB* genes were used. All *cdtB*⁺ strains also showed cytotoxicity to HeLa cells, while none of the *cdtB*⁻ strains

Table 3. Histopathological changes in tissues of the small and large intestines of mice challenged with CDT⁺ and CDT⁻ *C. jejuni* culture supernatant

0, Normal histology; +, mild; ++, moderate; +++, severe inflammatory changes. See text for details.

Strain	Histological analysis			
	Stomach	Jejunum	Ileum	Colon
CDT⁺				
837	++	++	++	+++
556	+	++	++	+++
728	+	+	++	+++
726	0	+	+	++
314	0	+	++	++
CDT⁻				
356	0	0	0	+
130	0	0	0	0
584	0	0	0	+
978	0	0	0	0
701	0	0	0	0

did so. The greater level of adherence, invasion and cytotoxicity of *cdtB*⁺ versus *cdtB*⁻ *C. jejuni* strains indicates the role of CDT as a virulence marker of this pathogen. Different cell lines such as CHO, J774, Int 407 and Vero have been used to determine the virulence properties of *C. jejuni*, with variable results. One of the important explanations for this variation was that the cytotoxic effects on HeLa cells were more prominent in freshly seeded cells than in semi-confluent monolayers (Lee *et al.*, 2000). Freshly seeded cells may undergo active growth and protein synthesis and therefore may be more susceptible to the toxin. In the present study all the *cdtB*⁺ strains caused rounding in freshly seeded HeLa cells, suggesting that HeLa cells (freshly seeded) are good markers for detection of cytotoxic activity of *C. jejuni* strains.

CDT induces various types of responses (such as elongation, rounding and lethality) in HeLa cells and the mechanism(s) responsible for these responses is (are) still not known. Various *in vivo* models have been described to investigate the mechanism of pathogenesis associated with *C. jejuni* infection. In the present study, we chose the suckling mouse model because it is economical and various types of response can be monitored in the whole animal. We followed the protocol recommended by Okuda *et al.* (1997), who found that a 10 h starvation period of mice was found essential to obtain reproducible results. Watery secretion was observed in 6 of the 10 mice inoculated with supernatant having CDT activity while none of the animals administered supernatant lacking CDT activity had diarrhoea. These six mice died within 24 h, after commencement of diarrhoea, and were dissected immediately to collect tissues. All other mice were sacrificed at 48 h.

Although diarrhoea was not observed clinically in 4 of the 10 mice in the CDT⁺ group, remarkable changes in

pathology were observed in tissues of all mice administered supernatant of CDT⁺ *C. jejuni* strains. Panmural inflammation with mucosal denudation and necrosis affecting the jejunum, ileum and colon was observed in all cases. The mice inoculated with supernatant lacking CDT activity only had mild inflammation in the descending colon, while the rest of the gastrointestinal tissues had normal histology. Similar studies have reported that CDT-producing *E. coli* and *Campylobacter* spp. induced inflammatory responses in the small intestines of rabbits (Bouzari *et al.*, 1992) and rats (Johnson & Lior, 1988), respectively. However, Okuda *et al.* (1997) reported that the tissues of the small intestines remained apparently intact. The discrepancy in the results may be due to differences in the sensitivities of the animals. Literature suggests that heat-stable and heat-labile *E. coli* enterotoxin and cholera toxin stimulate the mucosal cells of the small intestine through enzymic actions without tissue damage (Kaper *et al.*, 1995; Knoop & Owens, 1992; Spangler, 1992). This appears to be the first study demonstrating biological activity of culture supernatant of CDT⁺ and CDT⁻ *C. jejuni* in the suckling mouse model. We also for the first time observed histopathological evidence of damage in the stomach and jejunum due to CDT.

In conclusion, the *cdtB* gene was more frequently present in *C. jejuni* than in *C. coli*. The presence of *cdtB* in *C. jejuni* was associated with increased adherence to, invasion of and cytotoxicity towards HeLa cells. The major pathological changes in the colons of mice administered supernatant containing *C. jejuni* CDT suggest that CDT is an important virulence attribute and the colon is its major target site.

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