Virulence properties of Campylobacter jejuni isolates of poultry and human origin

Kim Van Deun,1 Freddy Haesebrouck,1 Marc Heyndrickx,2 Herman Favoreel,3 Jeroen Dewulf,4 Liesbeth Ceelen,1 Linn Dumez,1 Winy Messens,2 Saskia Leleu,2 Filip Van Immerseel,1 Richard Ducatelle1 and Frank Pasmans1

1Department of Pathology, Bacteriology and Avian Diseases, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
2Institute for Agricultural and Fisheries Research (ILVO), Technology and Food Unit, Brusselsesteenweg 370, 9090 Melle, Belgium
3Department of Virology, Parasitology and Immunology, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
4Department of Reproduction, Obstetrics and Herd Health, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

Correspondence
Kim Van Deun
kim.vandeun@ugent.be

Received 16 April 2007
Accepted 25 June 2007

Campylobacter jejuni is one of the leading causes of food-borne gastroenteritis. Because of the high prevalence of C. jejuni in poultry, poultry meat is considered a major source of C. jejuni infections for humans. However, it is not known whether all poultry-associated C. jejuni strains are capable of causing disease in humans. Four different virulence properties of C. jejuni strains were compared between 20 poultry isolates and 24 human isolates. Strains were chosen based on their PFGE pattern to represent a heterogeneous population. The isolates were compared for their ability to invade and induce interleukin-8 (IL-8) production in T84 cells, their production of functional cytolethal distending toxin (CDT) using HEp-2 cells, and their sodium deoxycholate resistance. All four virulence factors were present among strains of human and poultry origin, with strong differences observed among strains. For invasion and IL-8 induction, no difference was observed between the two populations. However, on average, human isolates arrested more HEp-2 cells in their cell cycle than did the poultry isolates (P = 0.041), suggesting higher CDT production by the former. The ability to survive 16 000 μg sodium deoxycholate ml⁻¹ was significantly more pronounced (P = 0.006) among human isolates than poultry isolates, although all strains possessed the cmeABC operon. These data suggest that all four virulence properties are widespread among C. jejuni isolates, but that a higher degree of bile-salt resistance and more pronounced CDT production are associated with strains causing enteritis in humans.

INTRODUCTION

Campylobacter jejuni is recognized as one of the most common causes of human gastrointestinal disease in industrialized countries, and is frequently associated with the handling and consumption of contaminated poultry meat and cross-contaminated food products (Hänninen et al., 2000; Pearson et al., 2000; Wingstrand et al., 2006). A study in the UK showed that 80% of retail poultry is contaminated with C. jejuni (Corry & Atabay, 2001), and approximately 39% of the broiler flocks tested in a Belgian study conducted during 1998 and 2000 were positive for C. jejuni (Herman et al., 2003). This emphasizes the importance of poultry as a reservoir and source of C. jejuni infection.

Several virulence factors are considered to be important for the induction of gastroenteritis, such as resistance to bile salts (Lin et al., 2003), invasion of epithelial cells (Russell et al., 1993) and cytolethal distending toxin (CDT) production (Konkel et al., 2001). Cell invasion could result in cellular injury, leading to reduced absorptive capacity of the intestine, whereas CDT production is important for interleukin-8 (IL-8) release by intestinal cells in vitro (Hickey et al., 1999, 2000) and thus plays an important role in the host mucosal inflammatory response.

Although poultry meat is considered an important source of C. jejuni infections for humans, it is not known whether all poultry-associated C. jejuni strains are capable of
causing disease in humans. We therefore compared the capability for epithelial cell invasion, CDT production, bile-salt resistance and IL-8 induction of poultry and human isolates.

**METHODS**

**Experimental animals.** Day-of-hatch White Leghorn Chickens (Charles River Laboratories) were kept in brooder batteries and provided with food and water *ad libitum*. Husbandry, euthanasia methods, experimental procedures and biosafety precautions were approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University. Prior to use, chicks were screened for the presence of *Campylobacter* in the faeces. All of the chicks tested proved to be negative for *Campylobacter*.

**Bacterial strains.** In this study, 20 *C. jejuni* strains of poultry origin and 24 clinical isolates (kindly provided by Professor Peter Vandamme, Ghent University) were used. The choice of strains was based on their genotypic heterogeneity, which was determined by PFGE with *KpnI* and *SmaI* (Amersham Biosciences) according to the PulseNet USA protocol (http://www.cdc.gov/pulsenet/protocols/campy_protocol.pdf). PFGE profiles were clustered with BioNumerics software (version 4.0, Applied Maths) using the Dice coefficient and the unweighted pair group method using arithmetic averages (UPGMA) clustering algorithm. PFGE clusters were defined on a similarity level of 80%. All strains were routinely cultured in Nutrient Broth No. 2 (*Oxoid*), supplemented with *Campylobacter*-specific growth supplements (SR117 and SR0232, Oxoid) and 5% lysed horse blood (E&O Laboratories) at 42°C under microaerobic conditions (5% O₂, 5% CO₂, 5% H₂, 85% N₂). For certain experiments, horse blood was omitted. Care was taken to pass the strains no more than five times *in vitro*. Before use in virulence assays, all *C. jejuni* strains were passed through day-old specific pathogen-free (SPF) White Leghorn Chickens. Chicks were orally inoculated with 2 x 10⁷ organisms in 0.2 ml PBS. Controls were sham-inoculated with PBS alone. At 24 h post-inoculation, cloacal swabs were streaked on modified charcoal cefoperazone deoxycholate agar plates (mCDDA; CM0739, Oxoid) containing *Campylobacter*-specific growth supplements (SR0155 and SR0232, Oxoid) for 48 h at 42°C under microaerobic conditions. Bacteria were collected by washing plates with PBS, and the aliquoted suspension was stored in horse blood at −80°C.

For the CDT assay, bacterial cell lysates were prepared by sonication. Bacterial cultures were grown in 40 ml selective broth with 5% lysed horse blood for 19 h at 42°C in a microaerobic atmosphere to a density of 5 x 10⁴ c.f.u. ml⁻¹ and harvested by centrifugation at 900 g for 20 min at 4°C. The bacteria were washed once with 3 ml PBS and resuspended in 1 ml PBS. The pellet was sonicated on ice by eight 30 s pulses with an XL 2015 Sonicator (Misonix) followed by centrifugation at 6000 g for 10 min at 4°C. The supernatant was filter-sterilized by subsequent passage through 0.45 and 0.20 μm pore-size filters (IWAKI, International Medical) and stored at −80°C.

**Cell lines.** The HEP-2 cell line was obtained from the European Collection of Cell Cultures (ECACC). Cells were grown in Eagle’s Minimum Essential Medium (EMEM; Gibco, Invitrogen) supplemented with 10% fetal calf serum, 1% glutamine, 1% non-essential amino acids and, unless stated otherwise, 100 U penicillin ml⁻¹ and 0.1 mg streptomycin ml⁻¹ (Gibco) at 37°C in a 5% CO₂ atmosphere.

The human colon carcinoma cell line T84 (ECACC) was grown in 44% Dulbecco’s Minimal Essential Medium (DMEM), 44% F-12 (Gibco), 10% fetal calf serum (Integro), 1% l-glutamine, 100 U penicillin ml⁻¹ and 0.1 mg streptomycin ml⁻¹ at 37°C in a 5% CO₂ atmosphere.

**Invasion of T84 cells by *C. jejuni* strains of poultry and human origin.** T84 cells were seeded in 96-well plates at 1 x 10⁴ cells per well. After 78 h, the plates were centrifuged at 500 g for 10 min at 37°C to enhance bacterial contact with the cell monolayer. The plates were incubated for 3 h at 37°C in a 5% CO₂ atmosphere. Cells were washed with Hank’s Buffered Salt Solution (HBSS; Gibco) with Ca²⁺ and Mg²⁺ to remove non-invaded bacteria. To kill extracellular bacteria, 100 μg gentamicin ml⁻¹ (Gibco) was added to the wells. This concentration proved to be lethal for all strains tested. Plates were further incubated for 2 h at 37°C in a 5% CO₂ atmosphere. Gentamicin was washed away with HBSS without Ca²⁺ and Mg²⁺. Intracellular bacteria were released from T84 cells with 0.25% sodium deoxycholate and titrated on mCDDA plates.

**CDT production by poultry and human isolates.** The CDT production of *C. jejuni* isolates was determined by the assessment of the number of HEP-2 cells blocked in the G2 phase after exposure to *C. jejuni* lysate (Young et al., 2000). The DNA content of HEP-2 cells incubated with the *Campylobacter* cell sonicates was determined quantitatively by flow cytometry. HEP-2 cells were seeded at a density of 1 x 10⁵ cells ml⁻¹ in 25 ml culture flasks. The cells were treated with 100 μl bacterial sonicate and incubated for 72 h at 37°C with 5% CO₂. Sonicates from *C. jejuni* NCTC 11168 were used as a positive control (Purdy et al., 2000). Negative controls were treated likewise, but incubated with 100 μl PBS. After incubation, cells were washed once with medium without antibiotics and trypsinized. The cells were collected in a Falcon tube and pelleted by centrifugation at 1000 g for 5 min at 37°C. DNA was stained according to Chien et al. (2000). Briefly, the pellet was resuspended in 0.5 ml staining solution (3% PEG, 4 μg propidium iodide ml⁻¹, 9 U RNase A ml⁻¹, 0.1% Triton X-100, 0.0001% BSA, in 4 mM sodium citrate) for 20 min at 37°C, followed by the addition of 0.5 ml salt solution (3% PEG; 4 μg propidium iodide ml⁻¹, 9 U RNase A ml⁻¹, 0.1% Triton X-100, 0.0001% BSA, in 0.4 mM NaCl) and the mixture was stored for 1.5 h at 4°C in the dark. The DNA content was analysed using a FACScalibur flow cytometer (Becton-Dickinson) and data acquisition was performed using CellQuest software. For each experiment, 10⁴ cells were counted.

**Presence of the virB11, cdtA, cdtB and cdtC genes and the CmeABC multidrug efflux pump in human and poultry isolates of *C. jejuni*.** The presence of the virB11 gene as a marker for the plasmid pVir, the genes involved in CDT production cdtA, cdtB and cdtC, and the cmeABC genes for the multidrug efflux pump, was assessed using PCR. Bacterial chromosomal DNA was isolated from an overnight liquid culture without horse blood, using the DNeasy Tissue kit (Qiagen) according to the manufacturer’s instructions.

Primers (Operon Biotechnologies) used in this study and their sources are listed in Table 1. PCR was performed in a volume of 10 μl containing 0.03 U μl⁻¹ Polymerase Taq Platinum, 1 × PCR buffer, 3 mM MgCl₂ (Invitrogen Life Technologies), 40 μM dNTP (Qiagen), 0.5 pmol μl⁻¹ primers and 20 ng genomic DNA. Annealing temperature conditions varied according to the set of primers used. PCR products (3 μl) were separated by electrophoresis in an agarose gel containing 1.5% agarose (Boehringer Mannheim) in 1 x Tris-acetate/EDTA (TAE) buffer, pH 8, and stained with ethidium bromide. Gels were visualized using Image Master VDS (Pharmacia Biotech).

**Resistance to deoxycholate of *C. jejuni* strains of poultry and human origin.** MICs were determined using a modification of the
method described by Lin et al. (2002). Briefly, Campylobacter strains were plated on plates containing twofold dilutions of sodium deoxycholate (Sigma) between 1000 and 16 000 μg ml⁻¹ in mCCDA. Overnight cultures supplemented with horse blood were spotted at a density of 1 × 10⁸ bacteria, and the presence of growth was determined after 48 h at 42 °C under microaerobic conditions.

Induction of IL-8 production by T84 cells exposed to C. jejuni strains of poultry and human origin. T84 cells were seeded in 96-well plates at a concentration of 1 × 10⁵ cells per well. After 72 h the cells were inoculated with a bacterial culture grown overnight in broth without horse blood at an m.o.i. of 200. Wells were incubated with PBS as the negative control and with tumour necrosis factor (TNF)-α (200 ng ml⁻¹; Sigma) as the positive control. The plate was centrifuged at 500 g for 10 min to facilitate contact of bacteria with cells, and incubated for 24 h at 37 °C and 5 % CO₂. Supernatant was collected and centrifuged (2300 g, 10 min, 4 °C). The concentration of IL-8 in cell culture supernatant was measured by an ELISA (Amersham Biosciences) according to the manufacturer’s instructions.

Statistical analysis. Differences in occurrence of the virB11 gene in both populations were evaluated by means of Fisher’s exact test. Differences in T84 invasion, HEp-2 cell cycle arrest and IL-8 production were tested by means of one-way ANOVA. For the T84 results, log₁₀ transformation was performed to obtain normally distributed data. The capacity of the different strains to survive on different concentrations of sodium deoxycholate was analysed by means of Cox proportional hazard analyses. All statistical tests were performed in SPSS 14.0.

RESULTS AND DISCUSSION

Genotyping of poultry and human strains

Table 1. Primers used in this study

<table>
<thead>
<tr>
<th>Target and annealing temperature</th>
<th>Primer</th>
<th>Sequence (5’–3’)</th>
<th>Size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>cmeB gene, 55 °C</td>
<td>cmeBF</td>
<td>GGTACAGATCTGATGAAGCC</td>
<td>819</td>
<td>Lin et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>cmeBR</td>
<td>AGGATAAAGTGTGACGAAATT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cmeA gene, 55 °C</td>
<td>cmeAF</td>
<td>TTTGAGCTTGATGCTATGGCACTTTTTC</td>
<td>781</td>
<td>Lin et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>cmeAR</td>
<td>CTCGAGTCTTGAAGCTTGGCTAACC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cmeC gene, 53 °C</td>
<td>cmeCF</td>
<td>GGTTGGATCTTTATCGGAAAAAAA</td>
<td>641</td>
<td>Lin et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>cmeCR</td>
<td>TTTTTTTAAGTAGTTAATTTTTCTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>virB11 gene, 52 °C</td>
<td>virFW</td>
<td>GAACAGGAAAGTGGAAAAACTAGC</td>
<td>708</td>
<td>Bacon et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>virRV</td>
<td>TTCAGGATGGGCTATATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cdtA gene, 52 °C</td>
<td>cdtAFW</td>
<td>GGAATTTGGATTTGGGCTATACT</td>
<td>165</td>
<td>Pickett et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>cdtARV</td>
<td>ATCCAGAGGATAAGGGACATT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cdtB gene, 52 °C</td>
<td>cdtBFW</td>
<td>GTTAAATCCCTGCTATCAACCA</td>
<td>495</td>
<td>Pickett et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>cdtBRV</td>
<td>GTTGCGACTTGGAAAATTGCAAGGC-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cdtC gene, 52 °C</td>
<td>cdtCFW</td>
<td>TGGATGATAGGAGGATTTAACC</td>
<td>555</td>
<td>Pickett et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>cdtCRV</td>
<td>TGAGCATAACCAAAAGGAAG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To test the genetic stability of the isolates during passage, nine poultry isolates and nine clinical isolates recovered from cloacal swabs after passage were compared by PFGE to the original isolates that were used for oral inoculation of the chick. It was confirmed that six poultry isolates recovered after passage showed 100 % similarity to the original isolates by PFGE, while two isolates were slightly different (>90 % similarity) and one isolate was somewhat more different (80 % similarity). Four of the nine clinical isolates recovered after passage were identical to the original isolates by PFGE (>97 % similarity), while four isolates were slightly different (>92 % similarity) and one strain was more different (67 % similarity).

Prevalence of the virB11 gene is equal among poultry and human isolates

Since the virB11 gene is associated with invasiveness (Bacon et al., 2000), we screened the C. jejuni collection for its presence using PCR. The virB11 gene was present equally often in the poultry strains as in the human strains (P=0.20), suggesting that some poultry isolates are capable of invading the human intestine. It should be noted, however, that the prevalence of the virB11 gene was low:
the virB11 gene was present in only six of the 24 human isolates and two of the 20 poultry isolates.

C. jejuni strains from poultry and humans invade T84 cells equally

Invasion is generally considered to be important in pathogenesis, and a higher invasive capacity is generally reported for clinical isolates than non-clinical isolates (Fauchere et al., 1986; Konkel & Joens, 1989; Prasad et al., 1996; Tay et al., 1996). In our study, a total of 20 strains of poultry origin and 24 strains of human origin were compared with respect to their ability to invade T84 monolayers. Strains were considered invasive from log10(c.f.u. ml⁻¹)=1.0 and above. Data from four independent experiments for the poultry isolates and three independent experiments for human clinical isolates are summarized in Table 2. All but one isolate from poultry and one isolate of human origin were invasive. There was no significant difference (P=0.48) in mean invasiveness between the poultry isolates [mean ± SEM log10(c.f.u. ml⁻¹)=2.5 ± 0.1] and the human clinical isolates [mean ± SEM log10(c.f.u. ml⁻¹)=2.7 ± 0.1], supporting the findings of Lindblom & Kaijser (1995). However, strong differences in invasiveness between individual strains were noticed, and a greater proportion of human isolates than poultry isolates was highly invasive with log10(c.f.u. ml⁻¹) >3.5, but this tendency was not statistically significant.

Lysates from human C. jejuni strains arrest more HEp-2 cells in the G2 phase than poultry isolates

To investigate CDT production, lysates from 42 strains were tested for their ability to block the HEp-2 cell cycle. After 78 h incubation with bacterial sonicates or PBS, the DNA content of the cells was analysed by flow cytometry. Isolates which produced a cell cycle arrest of more than 16.9% (mean of negative controls plus 2 × SEM) were considered positive. The positive control strain NCTC 11168 caused a cell cycle arrest in 56.1 ± 3.8% of the HEp-2 cells, while the negative control treated with PBS arrested 7.5 ± 4.7% of the cells. On average, human isolates (n=23; mean ± SEM 55.3 ± 3.6%) were able to block more cells in their cycle progression (P=0.041) than the poultry isolates (n=19; mean ± SEM 43.5 ± 4.4%) (Table 3), confirming the findings on differences in toxigenicity between clinical and poultry isolates of Gilbert & Slavik (2004) and Prasad et al. (1996). For the extreme values, no differences in proportions between the poultry isolates and the human isolates could be found: one isolate from poultry (5.3%) and two isolates from humans (8.7%) were considered to be negative in their ability to produce functional CDT, while four poultry isolates (21.1%) and seven human isolates (30.4%) arrested more than 70% of the HEp-2 cells in their cell cycle progression. The proportion of human isolates (34.8%) which could induce between 50.0 and 70.0% of the HEp-2 cells to remain locked in the cell cycle was greater than the equivalent proportion of poultry isolates (5.3%).

All strains possessed the cdtA, cdtB and cdtC genes. It is indeed generally accepted that the cdt genes are widespread amongst poultry and human isolates (Bang et al., 2003; Eyigor et al., 1999; Rozynek et al., 2005). While our data suggest an association between CDT production and clinical outcome, two clinical isolates were CDT negative, while possessing the three cdt genes. The occurrence of CDT-negative strains in clinical isolates is in accordance with other observations (Abouon et al., 2005; Bang et al., 2001; Eyigor et al., 1999), and shows that CDT might not be the sole determinant in the final clinical outcome of C. jejuni infection in humans.

C. jejuni isolates from humans are more resistant to deoxycholate than poultry isolates

The ability of C. jejuni to reach the intestinal tract is associated with gastric-acid and bile-salt tolerance, and is a prerequisite for successful colonization and thus the induction of disease. Lin et al. (2003) showed a relationship between bile-salt resistance, colonization ability and the expression of the cmeABC operon. In our study, the prevalence of the cmeABC complex among the C. jejuni isolates was assessed using PCR. Although all 44 strains examined in this study possessed the three genes, strains were markedly different in their capacity to survive different concentrations of sodium deoxycholate. Human isolates of Gilbert & Slavik (2004) and Prasad et al. (1996) indeed generally accepted that the cdt genes are widespread amongst poultry and human isolates (Bang et al., 2003; Eyigor et al., 1999; Rozynek et al., 2005).

Table 3. CDT-induced cell cycle arrest in the HEp-2 cell line 78 h after exposure to bacterial lysates of C. jejuni strains of poultry and human origin

<table>
<thead>
<tr>
<th>Type of strain</th>
<th>Number (percentage of the total sample) of strains that induced G2 arrest of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;16.9 %</td>
</tr>
<tr>
<td>Poultry isolates (n=19)</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>Human isolates (n=23)</td>
<td>2 (8.7)</td>
</tr>
</tbody>
</table>

*Mean log10(c.f.u. ml⁻¹).*

http://jmm.sgmjournals.org

Downloaded from www.microbiologyresearch.org by
On: Wed, 02 Jan 2019 20:43:46
IP: 54.70.40.11

1287
isolates appeared to be significantly more resistant to bile salts than the poultry isolates ($P=0.006$) (Fig. 1), with $85\%$ of the human strains surviving $16\,000\,\mu g$ sodium deoxycholate ml$^{-1}$ compared to $54\%$ of the poultry isolates. Increased bile-salt tolerance might thus predispose to clinical campylobacteriosis in humans.

**C. jejuni** strains from poultry and humans induce IL-8 secretion by T84 monolayers to an equal extent

Because invasion and CDT production are two mechanisms which are held to be responsible for the induction of IL-8 release (Hickey et al., 1999, 2000), we examined the capacity of the two **C. jejuni** populations to elicit an IL-8 response in T84 cells. Data from three independent experiments are shown in Table 4. Strains inducing more than the mean of the negative control plus $2\times$ SEM (346.0 pg ml$^{-1}$) were considered to be positive in their ability to induce IL-8 production. The positive TNF-$\alpha$ (200 ng ml$^{-1}$) control induced a mean of 899.3 ± 57.0 pg IL-8 ml$^{-1}$. All strains mediated IL-8 production from the T84 monolayers. Although isolates of poultry origin ($n=18$; mean ± SEM 643.9 ± 31.3 pg ml$^{-1}$) were not statistically different ($P=0.29$) in their ability to induce IL-8 production from the human clinical isolates ($n=24$; mean ± SEM 726.2 ± 23.5 pg ml$^{-1}$), it was notable that more human isolates (66.7%) induced an IL-8 response between 600 and 700 pg ml$^{-1}$ than did poultry isolates (33.3%). In both **C. jejuni** populations, highly specialized strains exist: the lysate of the poultry strain KC 44 arrests only a small percentage of HEp-2 cells and is a poor invader, but causes the highest IL-8 response. Likewise, the human strain R-27478 induces an IL-8 response, but is a low CDT producer and invades T84 cells poorly. In contrast, two highly invasive and CDT-producing strains elicit the IL-8 response only moderately.

**Table 4.** IL-8 induction by T84 monolayer after 24 h exposure to **C. jejuni** isolates of poultry and human origin at an m.o.i. of 200

<table>
<thead>
<tr>
<th>Type of strain</th>
<th>Number (percentage of the total sample) of strains that induce:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;600*</td>
</tr>
<tr>
<td>Poultry isolates ($n=18$)</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td>Human isolates ($n=24$)</td>
<td>3 (12.5)</td>
</tr>
</tbody>
</table>

*$\mu g$ IL-8 ml$^{-1}$.

**Conclusion**

In this study, no correlation between PFGE type and phenotype could be observed, nor between PFGE type and the virulence properties themselves. The appearance of low CDT-producing and poorly invading strains, which are nevertheless able to elicit an IL-8 response, raises questions about the strategies employed by **C. jejuni** to cause pathology. We conclude that based on invasiveness and the ability to induce IL-8 production, all poultry isolates studied should be considered capable of causing human enteritis, and that enhanced CDT production and bile-salt resistance might predispose to **C. jejuni** clinical infections in humans.

**ACKNOWLEDGEMENTS**

This study was conducted under the Federal Public Service for Health, Food Chain Safety and Environment project (R-04/002-CAMPY). We thank Professor Peter Vandamme (Ghent University) for providing us with the human clinical **C. jejuni** isolates. The technical assistance of Marleen Foubert, Nathalie Van Ryselbergh, Gunter Massaer, Steven De Tollenaere and Daisy Guldentops was greatly appreciated.

**REFERENCES**


