Detection of cytolethal distending toxin (cdt) Genes in Campylobacter jejuni and Campylobacter coli isolated from the intestinal of commercial broiler chickens, turkey and quail of Iran

Shojaei Kavan, R.1, Hassanzadeh, M.1, Bozorgmehri Fard, M.H.1, Pourbakhsh, S.A.2, Akhondzadeh Basti, A.3, Barin, A.4, Ashrafi, I.5

1Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Iran
2Razi Vaccine and Serum Research Institute, Karaj, Iran
3Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Iran
4Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Iran
5Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Iran

Abstract:

BACKGROUND: Campylobacter jejuni and Campylobacter coli are zoonotic bacteria which are frequently associated with human diarrhea. Sharing of the cytolethal distending toxin (cdt) genes in Campylobacter is common and is considered species specific. OBJECTIVES: In this study we focused on detecting the presence of cdt gene in C. jejuni and C. coli isolated from broilers, turkeys and quails of Iran. METHODS: Cecal samples were randomly collected from 240 broiler chickens, 100 meat type turkeys and 100 quails after slaughtering. We used PCR as a method for detecting cdt genes. RESULTS: In broilers, 93% of 58 C. jejuni positive samples possessed cdt gene and in all cases the three different subunits of cdt genes were present. However, only 56% of 14 C. coli isolates in broilers had contained cdt genes, while one fourth having all three subunits present. In turkeys, around 65% of 34 C. jejuni positive samples had cdt gene present with 38% possessing all three subunits of cdt genes. But all 5 C. coli isolates had all three subunits cdt gene. In quails, 67% of 30 C. jejuni positive samples were identified by cdt gene, 20% of those possessed all three gene subunits. On the other hand, all 28 C. coli isolates of quails had cdt gene present while 36% of those held all three gene subunits. CONCLUSIONS: Our data is indicating the isolation, culture and cdt PCR amplification approaches in this study seemed to be efficient. However, the presence of different variation of Campylobacter cdt gene types in our sample isolates signifies the necessity of further functional gene studies to elucidate which gene type combinations result in encoding effective toxins.

Key words: broilers, turkeys, quails, cdt gene, Campylobacter jejuni

Correspondence
Hassanzadeh, M.
Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Iran
Tel: +98(21) 61117150
Fax: +98(21) 66933222
Email: mhzadeh@ut.ac.ir

Received: 5 November 2014
Accepted: 2 February 2015

Introduction

Campylobacter jejuni and C. coli are zoonotic bacteria that are frequently associated with human diarrhea in both developing and in desterilized countries. Among many animal species housing Campylobacter species in their tract, wild birds and poultry are the most...
important reservoirs (Blaser et al., 1983). Typing of bacterial isolates from different sources provides epidemiological information needed for infection control and contributes to risk assessment of Campylobacter transmission. In Campylobacter, specific virulence mechanisms are not fully elucidated, although flagella mediated-motility, adhesion to intestinal mucosa, invasion and production of enterotoxin and cytotoxin have been identified as possible virulence determinates (Konkel et al., 1997; Wassenaar, 1997). A number of putative virulence and toxin genes have been studied, including fla A and cad F genes which are involved in adhesion and colonization of the host’s intestine (Konkel, 1999). Ceu E genes seem to encode a binding-protein transport system for the siderophore enterochelin (Gonzales, 1997; Park and Richardson, 1995). Cytotolethal distending toxin (cdt) cluster consists of three adjacent genes of cdt A, B and C. Cdt protein is composed of cdt B as the enzymatically active subunit, and the two heterodimeric subunit cdt A and cdt C, responsible for the holotoxin binding to cell membrane (Lara-Tejero and Galán, 2001, 2002). Campylobacter species cytotoxin cause DNA lesions, chromatin fragmentation, and cytoplasm distension which arrest cell cycle in G2/M transition phase, and leads to progressive cellular distension and ultimately cell death (Lara-Tejero and Galán, 2002; Nesic and Stebbins, 2005; Pickett and Whitehouse, 1999).

The aim of this study was to examine the presence of subunit cdt A, B, and C genes in Campylobacter jejuni and Campylobacter coli strains isolated from intestinal of commercial broiler chickens, turkeys, and quails by conventional microbiological culture followed by PCR amplification.

Materials and Methods

Sample collection: Intestine samples (cecal samples) were randomly collected from 240 broiler chickens of 8 flocks, 100 meat type turkeys, and 100 meat type quails, each from 5 flocks at the slaughter houses. The ceca of birds were placed in sterile plastic bags and transported to laboratory on ice for processing in the same day.

Isolation and identification of C. jejuni and C. coli: We used Campylobacter Charcoal Differential agar (CCDA) plate as direct culture (Funbac media, 23122, Tehran, Iran). The ceca was aseptically opened and a loop full of contents was plated on to be cultured. The plate was incubated in micro-aerobic conditions (Co2 8%) at 42 Cº for 24-48 hour. Colonies suspected as being Campylobacter species were examined for cell morphology by optical microscope. Single colonies were picked and characterized to species level by their catalase reaction, ability to hydrolyze hippurate and indoxyl acetate according to standard procedures (On and Holmes 1992). Isolates were stored with glycerol 15% at 70 Cº until required for further investigations.

DNA extraction: We transferred a loop full of colonies from the CCDA into an Eppendorf tube containing 250 μl phosphate-buffered saline (PBS). After the tubes were vortexed, the suspension were kept in 95 Cº Ban Mary for 15 min and then centrifuged at 9000xg for 10 min. Consequently, we were able to disassociate DNA in the upper phase of centrifuged tube.

Identification of Campylobacter species using multiplex PCR: For species identification, PCR was performed initially with the universal Campylobacter 16s rRNA primer (Linton et al., 1996). All PCR positive samples were then subjected to a second PCR for differentiation of C. jejuni from C. coli (Table 1). For CampF/CampR primer (Sina colon, Tehran, Iran), the reaction was performed in a 25 μl solution, containing 0.5 ng DNA, 0.1 μM each of CampF and CampR primers, 2 units of Tag DNA polymerase (Sina colon, Tehran, Iran), 0.2 mM of each dNTP, 10 mM Tris-HCl
and 2.5 mM MgCl2. The amplification reactions were carried out using a Perkin Elmer 9600 thermo cycler with the following program: one cycle of 10 min at 95 °C, 35 cycles of 30 s at 95 °C, 1.5 min at 59 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C. For Campylobacter species used 0.2 μM of MDmapA1/MDmapA2 and COL3/ MDCOL2 primers. The amplification reactions were carried out using a Perkin Elmer 9600 thermo cycler with the same program described above. For the visualization of PCR products, 10 μl aliquots were subjected to electrophoresis in 1.5% agarose gel stained with ethidium bromide for 1.5 hour at 100 V, and viewed under UV light.

Detection of cdt genes from Campylobacter isolates by multiplex PCR: The multiplex PCR was used to detect the cdt A, B and C genes of C. jejuni and the cdt A, B and C of C. coli (Asakura et al., 2008) are summarized in table 2. The PCR protocol of Findik et al., (2011) was modified as described below. Our result prove that the concentration of the primer sets (Sina colon, Tehran, Iran) in the multiplex PCR reactions was appropriate, 40 μl of PCR reaction mix contained 0.2 mM dNTP mix and 1.0 U of Ex Taq DNA polymerase. PCR products were analyzed by 1.5% agarose gel electrophoresis. Bands were visualized and images were captured.

Results

In this study, PCR method was used not only for confirmation of genus and species but also for detecting of cdt subunits genes using three different primer sets in two different combinations (Tables 1 and 2). In first step, for genus detection of Campylobacter isolates, Campylobacter jejuni ATCC 29428 and Campylobacter coli ATCC 43478 was used as our positive control (Figures 1 to 3).

From 240 samples of broiler chickens, 72 (30%) thermophilic Campylobacter were iso-
58 of these isolates (81%) were *C. jejuni* and 14 (19%) were *C. coli*. Out of 58 *C. jejuni* isolates, 54 (93%) were *cdt* positive, which all of them had all three types of *cdt* A, B and C genes, but 4 isolates had no any of those subunit genes (Table 3). Between the 14 *C. coli* isolates, 8 (56%) isolates indicated at least one of those *cdt* A, B or C genes and 6 (44%) had no *cdt* gene. Out of 8 isolates 2 (25%) had all three types of *cdt* genes, 2 (25%) isolates had both *cdt* A and B, 2 (25%) isolates had only *cdt* A and 2 (25%) isolates had only *cdt* C (Table 3).

### Table 1. Primers used for identification genus and species of *Campylobacter* by polymerase chain and multiplex polymerase chain reaction.

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer Name</th>
<th>Sequence (5’–3’)</th>
<th>PCR Product Size</th>
<th>Specificity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S</td>
<td>CampF</td>
<td>AGTCTTGGCACTATGACCTAAG</td>
<td>408</td>
<td>Genus</td>
<td>Wangroongsarb et al, 2011</td>
</tr>
<tr>
<td>rRNA</td>
<td>CampR</td>
<td>ATATGCCAAATTGACCGTGTGTCG</td>
<td>589</td>
<td><em>C. jejuni</em></td>
<td>Stucki et al, 1995</td>
</tr>
<tr>
<td>MapA</td>
<td>MDmapA1</td>
<td>CTATTTATTTTTGAGTTGTTTG</td>
<td>462</td>
<td><em>C. coli</em></td>
<td>Gonzalez et al, 1997</td>
</tr>
<tr>
<td>CeuE</td>
<td>COL3</td>
<td>AATTGAAAGTTCTCACTATAG</td>
<td>589</td>
<td><em>C. jejuni</em></td>
<td>Stucki et al, 1995</td>
</tr>
<tr>
<td></td>
<td>MDCOL2</td>
<td>TGGATTATTTTGAGTTTGACG</td>
<td>351</td>
<td><em>C. coli</em></td>
<td>Gonzalez et al, 1997</td>
</tr>
</tbody>
</table>

### Table 2. Characteristics of PCR primers used for *cdt* genes in the study. (*= *C. jejuni*; **= *C. coli*).

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’–3’)</th>
<th>Target</th>
<th>PCR condition</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Cj-CdtAU2</td>
<td>AGGACTTGAACCTAATTTTC</td>
<td>Cj-<em>cdt</em> A</td>
<td>94 °C, 30s 55 °C, 30s 72 °C, 30s</td>
<td>631</td>
</tr>
<tr>
<td>*Cj-CdtAR2</td>
<td>AGGTGGAATGTTAAAAACCC</td>
<td>Cj-<em>cdt</em> B</td>
<td>94 °C, 30s 56 °C, 30s 72 °C, 30s</td>
<td>714</td>
</tr>
<tr>
<td>*Cj-CdtBU5</td>
<td>ATCTTTTACCTTTGTTCGCC</td>
<td>Cj-<em>cdt</em> C</td>
<td>94 °C, 60s 57 °C, 60s 72 °C, 60s</td>
<td>339</td>
</tr>
<tr>
<td>*Cj-CdtBR6</td>
<td>GCAAGCATTTAATTCGCCGC</td>
<td>Cc-<em>cdt</em> A</td>
<td>94 °C, 30s 55 °C, 30s 72 °C, 30s</td>
<td>329</td>
</tr>
<tr>
<td>*Cj-CdtCU1</td>
<td>TAGGGATATGACCGAAGAG</td>
<td>Cc-<em>cdt</em> B</td>
<td>94 °C, 30s 56 °C, 30s 72 °C, 30s</td>
<td>413</td>
</tr>
<tr>
<td>*Cj-CdtCR1</td>
<td>GCTTAATACAGTTACGATAG</td>
<td>Cc-<em>cdt</em> C</td>
<td>94 °C, 30s 55 °C, 72 °C, 30s</td>
<td>313</td>
</tr>
</tbody>
</table>

### Table 3. The occurrence of *cdt* gene subunits in *Campylobacter* species isolated from commercial broiler chickens, turkeys and quails. *= number / percentage of characters *cdt* gene subunits.

<table>
<thead>
<tr>
<th>Birds</th>
<th>Number of isolates (%)</th>
<th>Number / percentage of characters <em>cdt</em> gene subunits in thermophilic <em>Campylobacter</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Broilers</td>
<td>58 (81%)</td>
<td>54 (93%)</td>
</tr>
<tr>
<td>Turkeys</td>
<td>34 (87%)</td>
<td>13 (38%)</td>
</tr>
<tr>
<td>Quails</td>
<td>30 (52%)</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Total</td>
<td>122 (72%)</td>
<td>73 (59%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Birds</th>
<th>Number of isolates (%)</th>
<th>Number / percentage of characters <em>cdt</em> gene subunits in mesophilic <em>Campylobacter</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Broilers</td>
<td>14 (19%)</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Turkeys</td>
<td>5 (13%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Quails</td>
<td>28 (48%)</td>
<td>10 (36%)</td>
</tr>
<tr>
<td>Total</td>
<td>47 (28%)</td>
<td>17 (36%)</td>
</tr>
</tbody>
</table>
Of the 100 intestinal samples collected from turkeys, 39 were thermophilic Campylobacter positive. Among these positive, 34 isolates (87%) were C. jejuni and 5 (13%) were C. coli. Out of 34 isolated C. jejuni, 22 (65%) had at least one of the three subunit cdt genes. Between these, 13 (38%) isolates had all types of cdt A, B and C genes, 1 isolate had both cdt A and B, 1 isolate had cdt A and C, 1 isolate had cdt B and C, 1 isolate had only cdt A, 3 isolates (9%) had only cdt B and 2 isolates (6%) had only cdt C. Furthermore, all 5 C. coli isolates in turkeys had all types of cdt A, B and C genes (Table 3).

Among 100 samples collected from quails, 58 thermophilic Campylobacter were isolated. Out of those, 30 (52%) were C. jejuni positive and 28 (48%) were C. coli. Between 30 C. jejuni, 20 isolates were cdt positive, in which 6 of them (20%) had all three types of cdt genes, 10 isolates (33%) had cdt B and C simultaneously and 4 isolates (14%) had only cdt B. Out of 28 C. coli positive, 10 isolates (36%) had all three types cdt A, B and C genes, 4 (14%) had cdt A and B together, 10 (36%) had cdt A, 2 (7%) had cdt B and 2 (7%) also had cdt C (Table 3). Interestingly that both Campylobacter jejuni and Campylobacter coli were frequently recovered at the same time from individual examined birds.

Discussion

According to epidemiological studies taken place in Iran, the reported prevalence of Campylobacter infection in poultry of Isfahan was 56 % (Rahimi and Tajbakhsh, 2008). These reports in Shahrekord was 47 % (Rahimi and Ameri, 2011; Rahimi, 2013) but in Tehran, was 50 to 63 % (Taremi et al., 2006). In the other study in Tehran, the prevalence of Campylobacter on turkeys was 55 % and quails was 22 % respectively (Mirzae et al., 2011). The Campylobacter infection of poultry meat in other places of the world is also considerably high, for instance 92 % in turkey (Yildirim et al., 2005), 87 % in Poland (Wieczorek et al., 2012) and 64 % in Italy (Di Giannatale et al., 2012). Also in an Irish study on turkey and poultry meat, Campylobacter infection was reported 38 % and 50 % respectively (Whyte et al., 2004). In our study, the number of samples infected with Campylobacter jejuni is considerably higher than the ones infected with the Campylobacter coli (73 and 27 percent respectively). This figures are confirmed by similar earlier studies (Rahimi and Tajbakhsh, 2008; Son et al., 2007; Dipineto et al., 2010), however they differ with some reports (Di Giannatale et al., 2012). The proportion of the infection with these two bacterial species seems to be highly variable in different studies. Such difference might be a result of various avian infection in different area, sampling methods, different slaughter and processing approaches, seasonal of sample collection or sensitivity of statistical methods in these studies (Stern and Line, 1992; Wallace et al., 1997).

In the present study, regardless of avian sample type, 59% of all C. jejuni isolates and 36% C. coli isolates had all three sub-types of cdt genes (A, B and C). However in the study done by Dipineto et al. (2010) in Italy, 100 % C. jejuni isolates had all three types of cdt gene present. Authors also pointed that 100% of C. coli isolates had cdt B, 99% isolates showed cdt A and 99% showed cdt C positive. In the another report on Danish broilers, Bang et al. (2001) mentioned all cdt gene types were present in 99% isolates of C. jejuni and 100% isolates of C. coli. In the study performed by Van Deun et a., (2007) four pathogenicity factors of C. jejuni were compared between 24 human and 20 poultry isolates. The result of their study confirmed the presence of cdt genes in all studied isolates. Study done by Findik et al. (2011) on human, cattle, sheep, dog and poultry samples indicated that 76% of C. jejuni isolates were cdt A, B and C positive. While,
0.6%, 11% and 6% of their isolates were respectively cdt B, cdt C and cdt A negative. Among their isolates 4% showed only cdt B and 3% did not have any type of cdt genes present. According to Findik et al. (2011) prevalence of cdt gene types can be vary between 69 and 100% for cdt A, 92 and 100% for cdt B and 39 and 98% for cdt C. 

Recently, Ehsannejad et al. (2015) reported the virulence markers of cdt genes in fecal samples of pet birds of Iran. In their study, 20 thermophilic Campylobacter were detected from 8 different avian species. From 20 confirmed Campylobacter spp. 16 samples (80%) were C. jejuni positive and 4 (20%) were C. coli by species-specific PCR test. Furthermore, out of 20 detected Campylobacter, 13 (65%) harbored the various subunits of cdt A, cdt B and cdt C genes, and 7 (35%) were negative for all tested cdt genes. They concluded that the carriage rate of Campylobacter in different species of cage and/or in zoo birds was also high and confirmed that cdt genes may frequently be present in Campylobacter spp. 

The present study is indicated that cdt genes may frequently be present in Campylobacter spp, isolated from commercial birds, however the role of those in campylobacteriosis is still in question. Further study is needed to improve the understanding of the role of CDT in the pathogenesis of campylobacteriosis. Considering the importance of presence of cdt gene types in construction of an active cdt holotoxin, it is also recommended to take more gene functional approaches for future exploration of pathogenicity mechanism of thermophilic Campylobacter.

Acknowledgments

This research was supported by a grant from the Research Council of the University of Tehran.

References


Detection of cytolethal distending toxin (cdt)...

Shojaei Kavan, R.

شناسایی زن‌های سم‌کشنده تورمی سلولی (cdt) در کمپیلوباکتر زئوئنی و کمپیلوباکتر کلی جدایی‌شده از روده‌های گوشتی، بوقلمون و بلدرچین تجاری در ایران

روگاه شیمی‌گیاه‌شناس 1، محمد حسن زاده 2، محمد حسن بزرگمهری‌فرد 3، سید علی زرگمه‌پور 4، افیسین آخوندزاده‌بستی 5، عباس برین 6، ایرج اشرافی 7، افرین آخوندزاده بستی 8، سید علی پوربخش 9، سید علی پوربخش 10، محمد حسن بزرگمهری‌فرد 11، محمد حسن زاده 12، سید علی پوربخش 13، سید علی پوربخش 14، محمد حسن بزرگمهری‌فرد 15، محمد حسن زاده 16

مجله طب دامی ایران، 1394، شماره 9، دوره 1394

چکیده

زمینه مطالعه: کمپیلوباکتر کلی و کمپیلوباکتر زئوئنی، باکتری‌های زئوئنی هلستینی که در بسیاری از موارد سبب اسهال در انسان می‌شوند. زن‌های سم کشنده تورمی سلولی (cdt) در کمپیلوباکترها شایع و اختصاصی می‌باشد. هدف این مطالعه بررسی حضور زن‌های سم کشنده تورمی سلولی (cdt) در کمپیلوباکتر کلی و کمپیلوباکتر زئوئنی که از گله‌های جوجه‌گوشتی، بوقلمون و بلدرچین جدا نمودیم، برداخته‌ایم. روش کار: نمونه‌های جوجه‌گوشتی، بوقلمون و بلدرچین تهیه و پس از جداسازی باکتری با استفاده از پرداخته‌ایم. نتایج: در جوجه‌گوشتی، ۱۰۰ قطعه پرداخته و پس از جداسازی باکتری با استفاده از PCR از جنس جوجه‌گوشتی، ۱۰۰ قطعه پرداخته و پس از جداسازی باکتری با استفاده از PCR، ۱۴ نمونه کمپیلوباکتر زئوئنی جدایی‌شده، واجد قطعاتی از این ژن بودند، و از این پرنده واجد قطعاتی از این ژن بودند. در بوقلمون، ۵۶ نمونه کمپیلوباکتر کلی جدایی‌شده، واجد قطعاتی از این ژن بودند. در بلدرچین، ۶۵ نمونه کمپیلوباکتر کلی جدایی‌شده، واجد قطعاتی از این ژن بودند. در نتیجه: حرکت ژن‌های سم کشنده سلولی، باعث افزایش اسهال در جوجه‌گوشتی، بوقلمون و بلدرچین می‌شود. تفاوت‌های میان روش‌های مناسبی جهت بررسی cdt می‌باشد. اطلاعات بدست‌آمده در این مطالعه، جداسازی، کشت، PCR روش‌های مناسبی جهت بررسی cdt می‌باشد. تفاوت‌های موجود در حضور ژن‌های cdt در نمونه‌های بدست‌آمده هم، اهمیت عملکرد انزیم cdt در کنترل سایر ژن‌ها برمی‌تواند سی‌سالین انزیم را کنترل کند.

واژه‌های کلیدی: جوجه‌گوشتی، پرداخته، PCR، نمونه‌برداری، cdt

Email: mhzadeh@ut.ac.ir

نویسنده مسئول: تلفن: +98 (21) 66932323، تلفن ثابت: +98 (21) 66932323، تلفن ثابت: +98 (21) 66932323، تلفن ثابت: +98 (21) 66932323، تلفن ثابت: +98 (21) 66932323