ISOLATION AND MOLECULAR CHARACTERIZATION OF CAMPYLOBACTER SPP. IN NEWLY HATCHED POULTRY IN EGYPT

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Abstract
Campylobacteriosis is a bacterial zoonosis transmitted from animals and birds to human contacts causing acute bacterial gastroenteritis. The current study aimed to conduct a surveillance of campylobacters among newly hatched poultry. Isolation and characterization of Campylobacter spp. were done by both traditional culture and PCR techniques. A total of 300 samples were collected from chicks (n=100), turkey poults (n=100) and duckling (n=100). The highest rate of Campylobacter spp. was recorded in ducklings (27 %), followed by chicks (3 %), while turkey poults showed no recovery of campylobacters. The recovery of Campylobacter spp. points out the poor hygienic measures and sanitation in poultry flocks and lack of fence to prevent cross infection to new generations as well as the undercooked poultry products or the mishandling of raw poultry products is the most likely source of exposure to Campylobacter spp.

Key words: Campylobacter; C. jejuni; C. coli; poultry; turkey.

INTRODUCTION
Campylobacters are curved rods that were classified as vibrios for many years [1]. This infection occurs primarily in infants, elderly people, and patients with underlying disease. The disease is accompanied by fever, bloody diarrhea, headache and abdominal pain. A collective name for infectious diseases caused by members of these bacteria is called Campylobacteriosis[2]. The most important pathogenic strains belonging to the group of thermos-tolerant campylobacteria are C. jejuni and C. coli which cause serious complications related to acute bacterial enteric disease in humans worldwide [3,4].

On the other hand, Campylobacter spp. including C. jejuni and C. coli are common contaminants of poultry carcasses in poultry processing plants [5,6] as they preferentially inhabit the intestines of birds, including chickens, turkeys, quails, ducks, wild birds and even ostriches [7]. Contamination can also occur directly through air or bird to bird via equipment and water [8]. Different studies have demonstrated high prevalence of such pathogen in chickens, ducks and turkeys, ranging from 40% to 100% [9,10].

Laboratory diagnosis of Campylobacter including isolation and identification is expensive, laborious and time consuming. The organism is fastidious and grows slowly with specific
requirements in incubation conditions. However, PCR has increasingly been applied in the
detection and identification of Campylobacter spp. [11,12].
In Egypt, the disease is endemic, however, the epidemiology in animals and humans has not
been fully characterized [13]. Therefore, the aims of the current study are to compare between
conventional and molecular methods to detect the presence of Campylobacter spp. in newly
hatched imported poultry (chicks – ducklings – turkey poults), as well as to get an information
regarding the occurrence of campylobacters in such birds. This information could be useful to
reach a feasible diagnostic method and also for planning strategies for the prevention and
control of avian campylobacteriosis.

MATERIALS AND METHODS

Sampling
A total of 300 fecal swabs was collected from ducklings (n=100), chicks (n=100) and turkey
poults (n=100).

Isolation and identification
Isolation and identification of Campylobacter spp. from fecal material was performed according
to ISO 10272 [14]. Briefly, fecal swabs were inoculated in Bolton broth and incubated at 42°C
for 48 hours under microaerophilic condition with 10% CO₂ tension. After then, a loopful of
enrichment broth were plated on modified charcoal cefoperazone-deoxycholate agar (MCCDA)
(Oxoid, UK) and incubated in the same microaerophilic atmosphere. Suspected colonies of
Campylobacter were identified under phase contrast microscope for detection of characteristic
motility and morphological character. Campylobacter isolates were subcultured and identified
by biochemical tests described before [15,16,17].

Polymerase chain reaction (PCR)
- DNA extraction. DNA extraction from samples was performed using (BioPure
  Genomic DNA Isolation and Purification Kit, India). The extraction kit was done
  according to the manufacturer’s instructions.
- Oligonucleotide primers used in PCR
  Three pairs of primers were supplied from (Bio Basic Inc., Canada). They have specific sequence
  and amplify specific products as shown in table (1).
- PCR amplification
  The samples were subjected to different PCR cycles according to Wang et al. [12] determining
  genus Campylobacter and distinguishing between C. coli and C. jejuni. PCR reaction products
  were separated on 1.5% agarose gels, stained with ethidium bromide and visualized. C. coli
  strain (ATCC# 43478) and C. jejuni strain (ATCC# 33560) were included as positive controls.

RESULTS
The occurrence of Campylobacter spp. in the collected samples is detailed in table (2). A
comparison between culture results and PCR results are also shown in the same table. Briefly,
the highest rate of Campylobacter spp. was recorded in ducklings (27 %), followed by chicks (3
%). Turkey pouls showed no recovery of campylobacters. Table (3) shows the occurrence of C.
coli and C. jejuni among bird species.

DISCUSSION
Campylobacter spp. could cause serious complications related to acute bacterial enteric disease
in humans throughout the world [3,18]. Thermophilic campylobacters, including Campylobacter
jejuniand Campylobacter coli are intestinal commensals of domesticated birds. Very low doses of
thermophilic campylobacter cells are sufficient to colonize chicks and enter in a non-pathologic
(commensal) association within the intestine following colonization [19,20]Campylobacters
preferentially inhabit the intestines of birds, including chickens, turkeys, quails, ducks, wild
birds and even ostriches [7]. In addition, some epidemiological studies demonstrated high
prevalence of this microorganism in chickens, ducks and turkeys, ranging from 40% to 100%
[9,10]. In the current study, we have found that the rates of such organism in ducklings and

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chicks were 27 % and 3 %, respectively, while turkey poults showed no recovery of campylobacters.

On the other hand, some epidemiological studies have revealed a firm association between Campylobacter infections in humans and the handling and consumption of raw or undercooked poultry meat [21]. Not only poultry meat, but also exposure to poultry backyards could enhance the risk of campylobacter infection [13]. Herewith, in the same vein, our results reinforce the potential public health hazard when people handle ducks and chicks.

Moreover, we have isolated such pathogen from one day old birds, the isolation of Campylobacter spp in one day old was recorded by Shane [22]. Other articles [17,23] suggested that vertical transmission of Campylobacter strains in birds could be suspected and therefore, early infection might be detected.

In the present study, the overall occurrence of Campylobacter spp. was (30/300) 10 %, out of which were identified as 3 C. jejuni and 24 C. coli in ducklings and 3 C. coli in chicks. This result was in concordance with many other articles [24,25, 26] which confirmed that C. coli is the most prevalent spp. identified from chicken carcass samples.

The higher isolation rates of Campylobacter spp. may be attributed to poor hygienic measures and sanitation in poultry farms and lack of fence to prevent cross infection to new generations [27], as well as the undercooked poultry products or the mishandling of raw poultry products is the most likely source of exposure to Campylobacter spp.

Table (1): Primer sequences used in PCR assay and the expected sizes of the products

<table>
<thead>
<tr>
<th>Primer</th>
<th>Target gene</th>
<th>Primer sequence(5’-3’)</th>
<th>size of amplified product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>23SF</td>
<td>23S rRNA targeting Campylobacterspp.</td>
<td>TATACCGGTAAGGAGTGCTGGAGATCAATTAACCTTCGAGCACC</td>
<td>650 bp</td>
<td>Wang et al., 2002</td>
</tr>
<tr>
<td>23SR</td>
<td></td>
<td>ATCAATTAACCTTCGAGCACC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CJF</td>
<td>hpo targeting C. jejuni</td>
<td>ACTTCTTTATGCTTGCTGCGCCAACAAAGAAGAAGC</td>
<td>323 bp</td>
<td></td>
</tr>
<tr>
<td>CJR</td>
<td></td>
<td>TCCAGCAATGTGTGCAATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCF</td>
<td>glyA targeting C. coli</td>
<td>GTAAACCAAGCTTATCGTGCCAGCAATG</td>
<td>126 bp</td>
<td></td>
</tr>
<tr>
<td>CCR</td>
<td></td>
<td>TCCAGCAATGTGTGCAATG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2) Occurrence of Campylobacter spp. by culture compared to PCR

<table>
<thead>
<tr>
<th>Bird species</th>
<th>Total no. of samples</th>
<th>No of positives by culture</th>
<th>No of positives by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duckling</td>
<td>100</td>
<td>27 (27 %)</td>
<td>27 (27 %)</td>
</tr>
<tr>
<td>Chicks</td>
<td>100</td>
<td>3 (3 %)</td>
<td>3 (3 %)</td>
</tr>
<tr>
<td>Turkey poults</td>
<td>100</td>
<td>0 (0 %)</td>
<td>0 (0 %)</td>
</tr>
<tr>
<td>Total no of samples</td>
<td>300</td>
<td>30 (10 %)</td>
<td>30 (10 %)</td>
</tr>
</tbody>
</table>

Table (3): Occurrence of C. jejuni and C. coli isolated from different bird species

<table>
<thead>
<tr>
<th>Types of bird</th>
<th>Campylobacterspp C. jejuni</th>
<th>C. coli</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duckling</td>
<td>3</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>Chicks</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Turkey poults</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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REFERENCES


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