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Research Paper

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

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Abstract

Campylobacteriosisis 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 bymembers 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% CO_2 tension. After then, a loopful of enrichment broth were plated on modified charcoal cefoperazonedeoxycholate 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 poults 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 jejuni*and *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

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 byShane [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

Primer	**Target gene**	**Primer sequence(5'-3')**	**size of amplified product**	**Reference**
23SF	23S rRNA targeting *Campylobacter*spp.	TATACCGGTAAGGAGTGCTGGAG	650 bp	Wang et al., 2002
23SR		ATCAATTAACCTTCGAGCACCG		
CJF	*hipO*targeting *C. jejuni*	ACTTCTTTATTGCTTGCTGC	323 bp	
CJR		GCCACAACAAGTAAAGAAGC		
CCF	*glyA* targeting *C. coli*	GTAAAACCAAAGCTTATCGTG	126 bp	
CCR		TCCAGCAATGTGTGCAATG		

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

Bird species	**Total no. of samples**	**No of positives by culture**	**No of positives by PCR**
Duckling	100	27 (27 %)	27 (27 %)
Chicks	100	3 (3 %)	3 (3 %)
Turkey poults	100	0 (0 %)	0 (0 %)
Total no of samples	300	30 (10 %)	30 (10 %)

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

Types of bird	***Campylobacter*spp**		**Total**
	C.jujeni	***C. coli***	
Duckling	3	24	27
Chicks	0	3	3
Turkey poults	0	0	0

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