

## *Campylobacter Jejuni* Infection in Broilers

Mohamed Shaker Abdel Hafez<sup>1\*</sup>, Nagwa Thabet El-Shraway<sup>2</sup>

<sup>1</sup>Poultry Diseases Department, Faculty of Veterinary Medicine, New-Vally University, Egypt

<sup>2</sup>Food Hygiene Department, Faculty of Veterinary Medicine, New-Vally University, Egypt

**\*Corresponding Author:** Mohamed Shaker Abdel Hafez, Poultry Diseases Department, Faculty of Veterinary Medicine, New-Vally University, Egypt. Email: [moemenassiut@hotmail.com](mailto:moemenassiut@hotmail.com)

**Abstract:** This study was aimed to assess the presence of *Campylobacter jejuni* infection and some of its virulence genes in broiler chickens in New Valley and Asyut provinces, Egypt. The study was conducted on 200 broiler chicken samples which obtained from 12 broiler flocks representing 60000 birds. One hundred intestinal samples and one hundred liver samples from birds (dead and live broilers suffering from diarrhea) during spring and summer seasons of 2016 were used for bacteriological and molecular examinations. The isolated and identified strains were examined for the presence of virulence factors (*VirB11*, *flaA* and *Iam* genes). Results showed that out of 200 intestinal and liver samples, *C. jejuni* was isolated in 51 (25.5%). The highest microbial load was in intestinal samples (26%) followed by liver samples (25%). In addition, the virulence genes of *C. jejuni* were identified in broiler chicken samples as *ViroB11* (10%), *flaA* gene (5%) and *Iam* gene (40%). In conclusion, this study revealed that *C. jejuni* may represent a threat to poultry production.

**Keywords:** broiler chickens, *Campylobacter jejuni*, virulence genes, PCR.

### 1. INTRODUCTION

Poultry houses can be contaminated by *Campylobacter* in many different methods from various environmental sources, making the control of flock colonization by *Campylobacter* a very challenging mission. At present, no single measure is completely effective in minimizing *Campylobacter* infections on poultry farms.

*Campylobacter* can break through biosecurity barriers and attack poultry houses, colonizing the chicken intestine and quickly multiplying in the intestinal mucosa. However, *Campylobacter* does not induce health or welfare troubles in chickens. *Campylobacter* spreads fast within broiler flocks and almost all birds will be infected within seven days (Garcia Ana *et al.*).

Poultry flocks can be infected by multiple sources; it is difficult to eliminate *Campylobacter* from poultry farms by biosecurity measures alone. The use of fly screens on poultry farms has been found to be an effective method to decrease the introduction of *Campylobacter* into broiler farms especially during summer period.

The exact mechanism by which *C. jejuni* is introduced into the poultry farm is still unclear, although a variety of important sources have been incriminated, including water (Pearson *et al.*, 1993), insects (Rosef and Kapperud 1983), farm personnel, and rodents (Annan-Prah and Janc

1988). Regardless of the source, once *C. jejuni* is introduced into a poultry flock, it spreads quickly and usually contaminating the entire flock (Shanker *et al.*, 1990).

One of the challenges associated with campylobacteriosis control is that *Campylobacter* behaves as a commensal micro-organism in healthy poultry flocks without causing any clinical symptoms. It is a normal inhabitant in the mucus layer of the cecum but does not penetrate the intestinal cells (Wael Abdelrahman 2014).

The persistence of *C. jejuni* during successive broiler flock rotations was suggested to be a result of the survival of the organism in many vectors as rodents and insects that were able to evacuate the house during cleaning and disinfection and then come back. However, no direct evidence mentioned to prove this opinion.

Other farm animals in broiler farms including pigs, cattle, sheep, and poultry other than chickens has been found to be associated with an increased risk of *Campylobacter* infection in broiler chickens.

In a follow up study, *C. jejuni* isolates from cattle were shown to have the same *flaA* type gene as the isolates from the broilers on the same farm.

*C. jejuni* is the major pathogen causing food borne diseases worldwide (Scallan *et al.* 2011).

Various studies demonstrated that certain bacterial factors are essential for the pathogenesis of *C. jejuni* including the motility and adherence to intestinal mucosa, capability of the bacteria to invade enterocytes as well as toxin production (Datta *et al.*, 2003 and Dasti *et al.*, 2010).

Some genes have been recognized as responsible for the expression of pathogenicity such as *flaA*, *virB11* and invasion associated markers (*iam*) genes which are genetic markers for *C. jejuni* (Nuijten *et al.*, 2000).

The main objectives of this study were to investigate the presence of *Campylobacter jejuni* in broiler chickens in the area of study and detection of their genes that have been recognized as responsible for the expression of pathogenicity.

**2. MATERIALS AND METHODS**

**Ethical Approval:** This study has been approved by the animal rights and ethical use committee of Assiut University.

**Area of Study:** The study was conducted in Assiut and New Valley provinces. This area of study represents about one half from the total Egypt area.

**PM Examination**

**Samples Collection:** A total of 200 samples were collected from 100 broiler chickens (dead and live birds suffering from diarrhea). The age of birds was 3-6 weeks. The samples were divided into 100 intestinal samples and 100 liver samples. This study was performed during the period between March and September 2016. Samples were sent to the laboratory and prepared for microbiological and molecular examinations.

**Samples Preparation:** Samples were prepared as the following; Ten grams from each sample were weighted under complete aseptic conditions, and transferred into sterile polyethylene bag containing 90 ml of sterile 0.1% peptone water (Oxoid). Samples were blended in a stomacher (Lab-blender, 400) for one minute to provide 10<sup>-1</sup> dilution.

**Microbiological Examination:** Total bacterial count was performed by adding 0.1ml of each

dilution to the following media in duplicate: Standard plate count agar (Oxoid) using drop technique. The plates were kept in the incubator at 35°C for 24 hours. Plates with distinct colonies counted between 30-300 colony were estimated as Colony Forming Units (CFU).

The isolation of *C. jejuni* was performed by streaking the dilutions on blood agar medium (Preston agar) (Oxoid), then incubated microaerobically at 42°C for 24-48 hours.

All the isolates were picked up and preserved on nutrient agar (Oxoid) and examined microscopically by Gram's stain to demonstrate the morphological arrangement and staining characters. Pure cultures of the isolates were biochemically identified according to Quinn *et al.*, (1994). Ten pure positive isolates of *C. jejuni* were selected for further enterotoxins and pathogenicity gene identification by PCR.

**PCR Protocol:** DNA was extracted from *C. jejuni* isolates from chicken samples using the QIA amp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. In brief, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis at 56°C for 10 minutes. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit. Primers used were supplied from (Biobasic, Canada) as showed in table (1). Primers were utilized in a 25µl PCR reaction containing 12.5 µl of 2X DreamTaq Green Mastermix kit (Fermentas, Germany), 1 µl of each primer of 10 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reactions were conducted in applied biosystem 2720 thermal cycler. The thermal cycle condition was used as initial denaturation at 94°C for 4 minutes, followed by 35 cycles of 94°C denaturation for 30 seconds, annealing (temperature differed as listed in table (1), for 30 seconds, and extension at 72°C for 45 seconds, followed by one cycle of final extension at 72°C for 5 minutes.

**Table1.** Primers sequences, target genes, amplicon sizes and annealing temperature of PCR reactions

Target gene	Primers sequences (5` to 3`)	Amplified segment (bp)	Annealing	Reference
<i>flaA</i>	AATAAAAATGCTGATAAAACAGGTG	855	53°C	Datta <i>et al.</i> , 2003
	TACCGAACCAATGTCTGCTCTGATT			
<i>VirB11</i>	TCTTGTGAGTTGCCTTACCCCTTT	494	53°C	
	CCTGCGTGTCTGTGTTATTTACCC			
<i>iam</i>	GCGCAAATATTATCACCC TTCACGACTACTACTATGCGG	518	46°C	Wieczorek <i>et al.</i> , 2012

The PCR products were separated by electrophoresis on 1.2% agarose gel (Applichem, Germany, GmbH) in 1X TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 10 µl of PCR products were loaded in each gel slot and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) were used to detect the fragment sizes. Control positive and control negative were used in each reaction. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

**3. RESULTS**

- 1. PM lesions** of dead broiler chickens were accumulation of fluid and gas or mucus in the intestines. Distention of intestines including ceca with watery fluid.
- 2. Total Prevalence of C. Jejuni among Broiler Chicken**

Results showed that the total prevalence of *C. jejuni* among chicken samples was 25.5%. The higher *C. jejuni* prevalence was in intestinal samples 26 (26%) followed by liver samples 25 (25%).

**3. Presence of Virb11, Flaa, and I am Virulence Genes among C. Jejuni Isolates from Chicken**

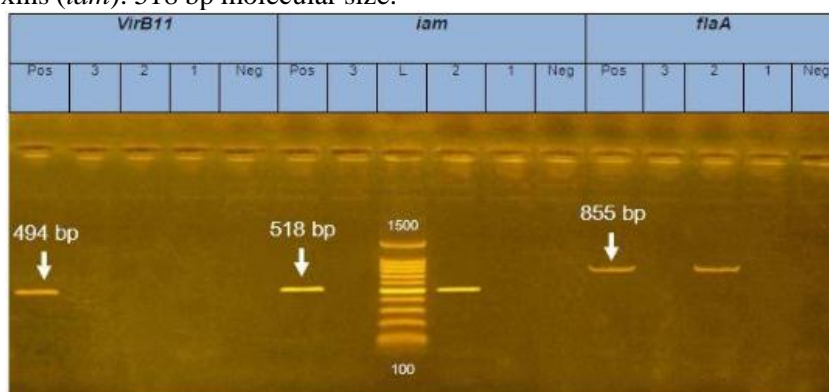
The prevalence of three main virulence genes *C. jejuni* from chicken intestinal (10 samples) and liver samples (10 samples) was reported in table (2) as following: the total prevalence of VirB11, flaA, and iam genes was 10%, 5%, and 40%, respectively. VirB11 gene was found in 2 (20%) in intestinal samples. flaA gene was detected in 1(10%) only in intestinal samples. The highest rate was detected in iam gene by a percentage of 5 (50%) in intestinal samples and in a lower rate in liver samples 3 (30%).

**Table2.** Prevalence of three virulence genes of among *C. jejuni* isolates from chicken samples

Samples	Virulence <i>C. Jejuni</i> genes		
	VirB11 No (%)	flaA No (%)	Iam No (%)
Intestinal samples	2 (20%)	1 (10%)	5 (50%)
Chicken liver	-	-	3 (30%)
Total No=20	2 (10%)	1 (5%)	8 (40%)

PCR amplification of the enterotoxins (*VirB11*) was 494 bp products of DNA extracted from *C. jejuni*, enterotoxins (*iam*): 518 bp molecular size.

while Enterotoxins (*flaA*) was 855bp of *C. jejuni* isolates from chicken samples respectively (figure 1).



**Figure1.** Agarose gel electrophoresis of specific dose-dependent amplification of *C. jejuni* enterotoxins (*VirB11*, *I am*, *flaA*) of chicken samples.

PCR amplification of the enterotoxins (*VirB11*) 494 bp products of DNA extracted from *C. jejuni*, enterotoxins (*iam*): 518 bp molecular size ladder Enterotoxins (*flaA*): 855bp of *C. jejuni* isolates from chicken samples respectively.

**4. DISCUSSION**

Chicken are susceptible to various bacterial agents which may be caused by a lack of hygiene, extensive exposure to the microbial agents and being carriers of these micro-organisms due to their genetic immunity. *C. jejuni* is one of these agents that can be isolated from poultry. (Abd El-Malek et al., 2010).

On commercial poultry farms, *Campylobacter* is rarely detected in birds less than 3 weeks of age. The reason for this lack of infection in young birds is unclear and may be related to various factors including the presence of maternally derived antibodies or differences in environmental or host-related conditions. Typically, the prevalence of *Campylobacter* rises as the birds grow and reaches the slaughter age for boiler chickens

*Campylobacter* infections in poultry usually produce no clinical symptoms of illness under natural conditions. Although, some studies recorded that experimental challenge of young chickens with *Campylobacter* can produce clinical signs in the form of watery/mucoid/bloody diarrhea, weight loss, or even mortality.

*C. jejuni* infect intestinal tract of several domestic poultry types frequently which be more sever in chicks which known as enter-invasive transient diarrhea resulting as watery droppings, hepatic focal necrosis, focal hemorrhage, jejunum distention or the absence of clinical signs. Infected bird shed *C. jejuni* after 2-3 weeks of infection (Shane, 2000). However, (Wagenaar *et al.* 2013) demonstrated that 100% of chickens infected asymptotically by *C. jejuni* in their intestinal tracts and shading the organism through feces.

The prevalence of *Campylobacter* in commercial broilers ranges from 20-90% (Shane, 2000 and Stern *et al.*, 2001). In Canada and South America, the prevalence of *Campylobacter* in commercial broilers ranges from 45-48% and 20-96%, respectively (Aho and Hirn, 1988 and Shane, 2000). In Europe, the proportion of commercial broiler flocks colonized with *Campylobacter* varies from 2.9% to more than 92% (Bouwknegt *et al.*, 2004; Evans and Sayers, 2000 and Hald *et al.*, 2000) with the lowest flock prevalence (2.9%) observed in Finland (Perko-Makela *et al.*, 2002). In other regions, the prevalence of *Campylobacter* within commercial broiler flocks ranges from 13.6% to 87% in Africa (Cardinale *et al.*, 2004), 24% to 54% in Asia (Newell and Fearnley, 2003). The obtained results of this study lay approximately in the middle area of the previous ratios.

While it is lower than the results of Saleha *et al.*, 1998 (42% in Australia) and the prevalence rate of infected flocks is 44-59% (Nauta and Havelaar, 2008).

As well as, the average *Campylobacter* prevalence rate on chicken at retail is 57% (Humphrey *et al.*, 2007).

Other study on the prevalence of *Campylobacter* in commercial broilers has shown that 85- 98% of commercial broiler flocks were colonized by *C. jejuni* (Avrain *et al.*, 2003).

The differences in the prevalence of *C. jejuni* could be attributed to the geographical area in this study. This study was conducted in a desert

area and very hot and dry weather might be a determinant of the prevalence rate.

A variety of PCR assays targeting genus- or species-specific sequences have been developed to detect and identify *Campylobacter* from poultry.

Some potential genetic markers of bacterial virulence have been identified. FlaA gene involved in adhesion and colonization, virB11 gene as pathogenic genes responsible for the expression of invasion and iam gene is associated with invasiveness and play a role in the transmission of *Campylobacter* and/or its adaptation to different hosts (Nuijten *et al.*, 2000, Sanad *et al.*, 2011; Young *et al.*, 2007 and Dasti *et al.*, 2010). In this study, the prevalence of VirB11 gene, flaA, and iam genes was 10%, 5%, and 40%, respectively in chicken gut and liver. Results of VirB11 gene in chicken was higher than that reported by many authors; 6.1% (Wiseczorek *et al.*, 2012), 7.3% (Gonzalez-Hein *et al.*, 2013) and 9.5%. (Datta *et al.*, 2003). The flaA gene was detected in 5% of chicken gut, which was much lower than previous reports, which reached even 100% (Wiseczorek *et al.*, 2012). The iam gene was detected in 40% of chicken liver and intestine which was higher than the previous study by Gonzalez-Hein *et al.*, (2013) (6.2%), whereas it was much lower than that detected by Wiseczorek *et al.*, (2012) who recorded 53.8%.

The detection of iam in *C. jejuni* isolated chickens, suggested a role for this marker in *C. jejuni* colonization. Moreover, it was proposed that the iam gene is not only essential for the colonization of the chicken gut but is also responsible for the induction of diarrhea (Korsak *et al.*, 2004 and Rozynek *et al.*, 2005). The detection of high rates of virulence genes among chicken samples referred to the potential hazard infection in poultry flocks and for consumers by eating undercooked or improperly cooked chicken and their livers.

In conclusion, this survey revealed that poultry was often contaminated with *C. jejuni* that might represent a threat to poultry production. Furthermore, several strains were positive for the several putative virulence marker genes which may represent a further hazard for health. Therefore, adequate biosecurity measures should be in consideration during the chicken cycles. The equipment cleanliness and chemical disinfectants are warranted.

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