

Research Article

Campylobacter Jejuni Infection and Virulence Genes in Broilers

Mohamed S Abdel Hafez*

Poultry Diseases Department, Faculty of Veterinary Medicine, New Valley University, Egypt

*Corresponding author

Mohamed S Abdel Hafez, Poultry Diseases Department, Faculty of Veterinary Medicine, New Valley University, El-Kharga, 72511, New Valley, Egypt, Email: Dr-Mohamed-Shaker@protonmail.com

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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 Assiut provinces, Egypt. The study was conducted on 200 broiler chicken samples which obtained from 12 broiler flocks representing 60000 birds. One hundred intestinal (caecal) 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

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. Nowadays, 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 gut and rapidly 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 [1].

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 still unclear, although a variety of important sources have been incriminated, including water [2] insects [3] farm personnel, and rodents [4] Regardless of the source, once *C. jejuni* is introduced into a poultry flock, it spreads quickly and usually contaminating the entire flock [5].

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 [6]

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* considering one of the most important pathogens causing foodborne diseases around the world [7]

Various studies demonstrated that the bacterial factors and the pathogenesis of *C. jejuni* including their intestinal mucosa habitat, which invading enterocytes in addition to its production of toxin [8] *C. jejuni* pathogenicity depend on presence of specific genes which is responsible such as flaA, virB11 and invasion associated markers (iam) genes which considered the genetic markers for *C. jejuni* [9]

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.

MATERIALS AND METHODS

Area of study

The study was conducted in Assiut and New Valley

governorates, that represent about 50% of the total landscape area of Egypt. Samples were obtained from 5 broiler farms located in Assiut City and 7 broiler farms in El-Kharga villages in New Valley governorate. The capacity of these farms were 2000 – 10000 broiler birds. Age of birds was 3 weeks (dead broilers) and 6 weeks (dead and slaughtered birds suffering from diarrhea).

PM examination

was performed for all samples and characteristic features were recorded.

Samples collection

A total of 200 samples were collected from 100 broiler chickens (dead and live birds suffering from diarrhea). The samples were divided into 100 intestinal (caecal) 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; 10 gm collected from each sample were collected aseptically, then 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 10-1 dilution.

Microbiological examination

Total bacterial count performed as following; 0.1ml of each dilution to Standard plate count agar (Oxoid), incubated at 35°C/24 hours, count colonies between 30-300 colony (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/24-48 hours. All the isolates preserved on nutrient agar (Oxoid) and examined microscopically by Gram's stain and biochemically [10] Ten pure positive isolates of *C. jejuni* were selected for further virulence genes identification by PCR.

PCR protocol

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

Electrophoresis of PCR products performed on 1.2% agarose gel (Applichem, Germany, GmbH) in 1X TBE buffer at 37°C 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) which detect the fragment sizes. Control negative and positive control were used in each reaction. Photographing the gel by a gel documentation system (Alpha Innotech, Biometra).

RESULTS

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.

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%).

Table 1: 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>	AATAAAAATGCTGATAAAAACAGGTG	855	53°C	Datta <i>et al.</i> , 2003
	TACCGAACCAATGTCTGCTCTGATT			
<i>VirB11</i>	TCTTGTGAGTTGCCTTACCCCTTTT	494	53°C	
	CCTGCGTGTCTGTGTTATTTACCC			
<i>Iam</i>	GCGCAAATATTATCACCC	518	46°C	Wieczorek <i>et al.</i> , 2012
	TTCACGACTACTACTATGCGG			

Table 2: 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%)

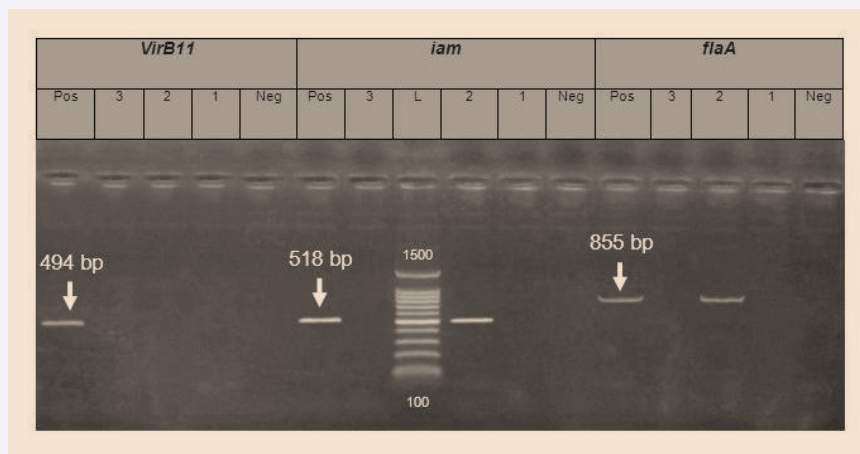


Figure 1 Agarose gel electrophoresis of specific dose-dependent amplification of *C. jejuni* enterotoxins (*VirB11*, *iam*, *flaA*) of chicken samples. PCR amplification of the enterotoxins (*VirB11*) 494 bp products of DNA extracted from *C. jejuni*, enterotoxins (*iam*): 518 bp molecular size ladder. Enterotoxins (*flaA*): 855bp of *C. jejuni* isolates from chicken samples respectively.

Presence of *VirB11*, *flaA*, and *iam* 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%).

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 855 bp of *C. jejuni* isolates from chicken samples respectively (Figure 1).

DISCUSSION

Chickens are susceptible to many bacterial agents and may be spread by a shortage of hygiene, frequent exposure to the bacterial agents which considered as carriers of these microorganisms due to their genetic immunity. *C. jejuni* is one of these agents that can be isolated from poultry. [11]. 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 live in the poultry intestinal tract which be more dangerous in chicks which called "enter-invasive transient

diarrhea" and appear as; hepatic focal necrosis, watery droppings, jejunum distention, focal hemorrhage or completely absence of clinical signs. Infected chicken shed *C. jejuni* within 2-3 weeks after infection [12]. However, [13], demonstrated that 100% of birds infected asymptotically by *C. jejuni* in their intestine, shedding the *C. jejuni* through feces.

The prevalence of *Campylobacter* in commercial broilers ranges from 20-90% [14] In Canada and South America, the prevalence of *Campylobacter* in commercial broilers ranges from 45-48% and 20-96%, respectively [12,15]. In Europe, the proportion of commercial broiler flocks colonized with *Campylobacter* varies from 2.9% to more than 92% [16-18], with the lowest flock prevalence (2.9%) observed in Finland [19] In other regions, the prevalence of *Campylobacter* within commercial broiler flocks ranges from 13.6% to 87% in Africa [20] 24% to 54% in Asia [21]. The obtained results of this study lay approximately in the middle area of the previous ratios. While, it is lower than the results of [22] (42% in Australia) and the prevalence rate of infected flocks is 44-59% [23].

As well as, the average *Campylobacter* prevalence rate on chicken at retail is 57% [24]. Other study on the prevalence of *Campylobacter* in commercial broilers has shown that 85- 98% of commercial broiler flocks were colonized by *C. jejuni* [25]. 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 important genetic markers of *C. jejuni* virulence have been detected. *FlaA* gene, *virB11* gene are pathogenic genes and perform the expression of invasion and *iam* gene is performed the invasiveness and transmission of *Campylobacter* and its adaptation to other hosts [26,27]. This research, prevalence of *VirB11* gene, *flaA*, and *iam* genes was 10%, 5%, and 40%, respectively in chicken intestine and liver. Results of *VirB11* gene in chicken was higher than that recorded by many authors; 6.1%

[28] 7.3% [29] and 9.5%. [30]. The *flaA* gene was detected in 5% of chicken gut, which was much lower than previous reports, which reached even 100% [28]. The *iam* gene was detected in 40% of chicken liver and intestine which was higher than the previous study by [29] (2013) (6.2%), whereas it was much lower than that detected by [28] 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 [31,32]. 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 study declared that chicken was usually contaminated with *C. jejuni* that might be discussed a threat to poultry production. In addition to, detection of several strains was positive for the several putative virulence marker genes which may represent a further hazard for health and resulted that, adequate biosecurity measures should be in consideration during the chicken cycles. The equipment cleanliness and chemical disinfectants are warranted.

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