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The Potential Role of Chicken Meat in Transmission of *Campylobacter Jejuni* to Consumers

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Abstract

Food-borne zoonotic diseases are a significant and widespread global public health threat. Campylobacter jejuni is a most common contaminant of chicken meat. This study was conducted to investigate the presence of C. jejuni and some of its virulence genes in chicken meat in New Valley, Egypt and determining their zoonotic impacts. A total of 300 chicken meat specimens were collected from 100 freshly slaughtered from breast (pectoral), thigh, and liver (100 of each) from (healthy and diarrheic) live bird markets and analyzed for C. jejuni burden using specific and selective nutrient media and molecular diagnosis. The identified strains were screened for virulence factors (VirB11, fla A and Iam) genes. Results revealed that that out of 300 meat samples, C. jejuni was detected in 74 (24.67%). The highest microbial load was in liver samples (26%) followed by pectoral (25%) then thigh muscle samples (23%). The virulence gene markers of C. jejuni was detected in chicken meat and liver samples as ViroB11 (2.27%), fla A gene (3.34%) and Iam gene (26.66%). Out of 50 diarrheic patients with food-poising signs 18 (36%) was positive for C. jejuni. The virulence markers genes in the human isolates showed that the prevalence of VirB11 gene, fla A, and Iam genes was 14.29 %, 71.43%, and 35.71%, respectively. In conclusion, this survey revealed that raw poultry meat available for consumers in ElKharga, Egypt was contaminated with zoonotic C. jejuni that might represent a threat to public health.

Keywords: *Chicken, Campylobacter Jejuni, Enterotoxine, Food Poisoning, PCR.*

Introduction

Foodborne diseases especially food poisoning is going up worldwide and becomes an important issue in public health impact by its fast contagiousness high morbidity and lethality. Food-borne zoonotic pathogens caused by consuming undercooked food or drinking water contaminated by pathogenic micro-organisms. Many of Food-borne zoonotic diseases are commonly found in the intestines of healthy food-producing animals and birds. Chicken is one of the major food-borne pathogens source by contaminated raw or undercooked chicken meat (Gwida & Elguhary, 2015).

Live bird markets are places where customers can buy domestic bird where either slaughter birds in the retail shops or at homes where minimal, if any, food safety standards or veterinary inspection are applied (Khalafalla *et. al.,* 2015). These markets related to traditional preference for consumption of freshly slaughtered poultry. Live poultry markets are an important part of the poultry supply chains in different parts of the world and may promote the emergence, spread and maintenance of livestock pathogens,

including zoonosis, In Egypt, the poultry meat trade depends mainly on that types markets that characterized by unhygienic slaughtering process, lack of marketing infrastructures.
C. jejuni is the major pathogen causing foodborne diseases worldwide (Scallan *et al.* 2011). The incident of C. *jejuni* is one of the most common causes of bacterial enteritis in human. Poultry products play an important role in transmission of Campylobacter bacteria to humans. *C. jejuni* contamination of poultry meat during processing has been reported (Berrang *et al.*, 2001). *Campylobacter* is most often detected in fresh broiler meat and in the EU the prevalence of these bacteria in broiler carcasses identified at the retail level varied from 3.1% to 58.8%, depending on the Member State (Anonymous 2010).

Several studies showed 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). Recently, some genes have been recognized as responsible for the expression of pathogenicity such as flagellin gene *fla A*, *virB11* and invasion associated markers (*Iam*) genes which are genetic markers for *C. jejuni* (Nuijten *et al.*, 2000).

It is necessary to know the extent to which the public is exposed to zoonotic *C. jejuni* infections via poultry meat as observed in slaughtered markets. The main objectives of this study were to assess the role of fresh chicken meat sold on the Egyptian live bird markets in the transmission of *C. Jejuni* entero-pathogene, detection of these genes have been recognized as responsible for the expression of pathogenicity and identifying its zoonotic implications.

Material and Methods

Ethical approval: This study has been approved by the animal rights and ethical use committee of Suez Canal and Assiut Universities.

The study area: This study was conducted in Elkharga which, is the capital of New Valley Governorate which is a part of the oasis which is located to the west of the Nile Valley, 232 kilometers to the South of Assuit Governorate. The New Valley Governorate represents about 45% from the total Egypt area.

Samples Collection: From farm birds, a total of 300 meat specimens were collected from 100 freshly slaughtered from 3 sites which were breast (pectoral) meat, thigh meat, and liver (100 of each) from New Valley public from (healthy and diarrheic) live bird markets. All data was recorded and samples were transported with minimal delay in an ice-box to the laboratory for microbiological and molecular examinations.

Samples preparation: Samples were prepared following the protocols of APHA, (1992) as 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^{-1} dilution.

Human samples: to identify the occurrence of *C. jejuni* food poisoning bacteria among human cases, 50 stool swabs were collected from the outpatients suffering from diarrhea and fever of Elkharga general hospital. Stool swab samples were examined for *C. jejuni* infections. Patients were interviewed and filled out a standardized questionnaire addressing the family's chicken consumption and purchasing and preparation conditions for chicken and their contacts with people having presented with an episode of diarrhea within 2-5 days after ingestion.

Microbiological examination: Total bacterial isolation 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 incubated at 35^{o}C/24hr. Plates with distinct colonies counted between 30-300 colony were enumerated as Colony Forming Units (CFU) and in Preston enrichment broth were incubated at 37^{o}C for 24 hours. After

enrichment, 0.1 ml of the broth was streaked onto modified Campylobacter selective agar base Cefoperazone Charcoal Desoxycolate Agar (Oxoid) containing antibiotic supplement (Oxoid). The plates were then incubated at 42ºC for 48 hours under microaerophilic conditions (5% O2, 10% CO2, and 85% N2) using Campylobacter gas generating kits (Oxoid) **(**Skirrow, 1977).

All the isolates were picked up and preserved on nutrient agar (Oxoid) and Preston broth (Oxoid) examined microscopically by Gram's stain to observe the morphological arrangement and staining reaction and pure cultures of the isolates were biochemically identified using catalase test, oxidase test, urea hydrolysis test, hydrogen sulphide (H2S) production, citrate utilization test and rapid hippurate hydrolysis test according to Quinn *et al.* (1994). Ten pure positive isolates for *C. jejuni* further enterotoxins and pathogenicity gene identification by PCR.

PCR detection: genomic DNA was extracted from the selected *C. jejuni* isolates from chicken and human samples using the QIA amp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis at 56°C for 10 min. 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 listed 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 performed in applied biosystem 2720 thermal cycler. The thermal cycle condition was used as initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C denaturation for 30 sec, annealing (temperature differed as mentioned in table 1), for 30 sec, and extension at 72°C for 45 sec. followed by on cycle of final extension at 72°C for 5 min.

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
flaA	AATAAAAATGCTGATAAAACAGGTG	855	53˚C	**Datta *et al.*, 2003**
	TACCGAACCAATGTCTGCTCTGATT			
VirB11	TCTTGTGAGTTGCCTTACCCCTTTT	494	53˚C	
	CCTGCGTGTCCTGTGTTATTTACCC			
iam	GCGCAAATATTATCACCC TTCACGACTACTACTATGCGG	518	46˚C	**Wieczorek *et al.*, 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 determine the fragment sizes. Control positive and control negative were included in each reaction. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

Results

Total prevalence of *C. jejuni* among chicken meat and stool samples from diarrheic human

As listed in table (2) and figure (1), results revealed that the total prevalence of *C. jejuni* among chicken samples was 24.67% whereas the total prevalence among diarrheic human stool samples was 28%. The higher *C. jejuni* prevalence was in liver samples 26 (26%) followed by pectoral muscles 25 (25%) and thigh muscle 23 (23%). All these percentage of chicken meat and liver were exceeding the Egyptian Organization Specification (EOS) and International levels of *C. Jejuni*: considered unfit for human consumption due to containing high microbial load of *C. jejuni* exceeding the permissible limits.

Table (2): Total prevalence of *C. jejuni* among chicken meat and stool samples from diarrheic human

Samples examined	Number of the examined samples	No (%)
Chicken Pectoral meat	100	25 (25%)
Chicken Thigh meat	100	23 (23%)
Chicken liver	100	26 (26%)
Total	**300**	**74 (24.67%)**
Human samples	**50**	**14 (28%)**

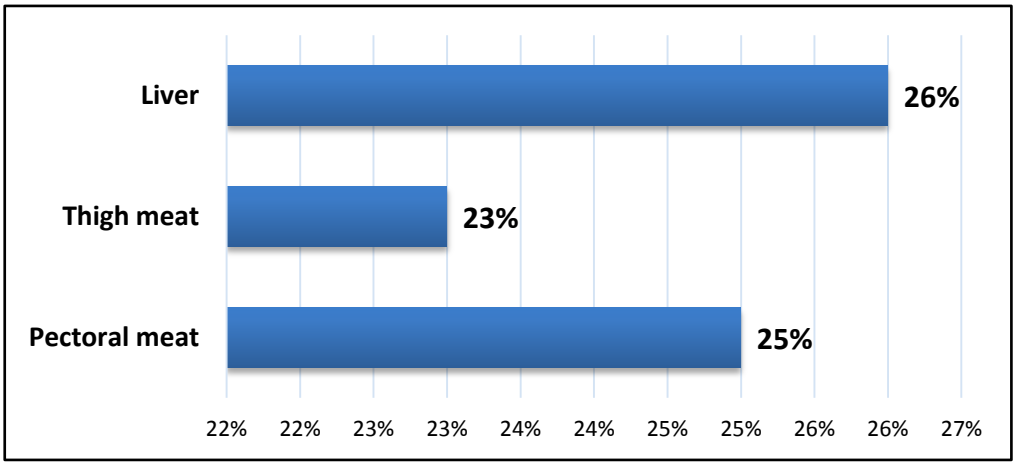

Figure (1): The percentage of chicken meat and liver samples exceeding the EOS standards of *C. Jejuni*.

Prevalence of VirB11, *fla A*, and iam virulence genes among *C. jejuni* isolates from chicken:

The prevalence of three main virulence genes among ten *C. jejuni* from chicken meat and liver samples was illustrated in table (3) and figure (2) as following: the total prevalence of VirB11, *fla A*, and iam genes was 6.67%, 3.34%, and 26.66%, respectively. VirB11 gene was detected in 2 (20%) in pectoral samples. *fla A* gene was detected in 1(10%) only in thigh meat. The highest rate was detected in iam gene by a percentage of 3 (30%) in pectoral and liver samples and 2 (20%) of the thigh muscles.

Prevalence of VirB11, Fla A, and iam virulence genes among human samples

As illustrated in table (3) and figure (2), out of 50 diarrheic patients with food poising signs, 18 (36%) for *C. jejuni*, *Among C. jejuni isolates*, the virulence gene markers were detected as following *VirB11*, 2 (14.29%), *fla A* 10 (71.43%), *Iam* 5 (35.71%). All human subjects were living in rural areas and all were buying chicken from public live bird markers.

Table (3): Prevalence of three virulence genes of among *C. jejuni* isolates from chicken meat and human stool samples

Samples	Virulence *C. Jejuni genes*		
	VirB11 **No (%)**	***Fla A*** **No (%)**	*iam* No (%)
Chicken Pectoral meat	2 (20%)	*ND	3 (30%)
Chicken Thigh meat	*ND	1 (10%)	2 (20%)
Chicken liver	*ND	*ND	3 (30%)
Total No=30	**2 (6.67%)**	**1 (3.34%)**	8 (26.66%)
Human samples	2/50 (14.29%)	10 (71.43%)	5 (35.71%)

***ND:** Not Detected

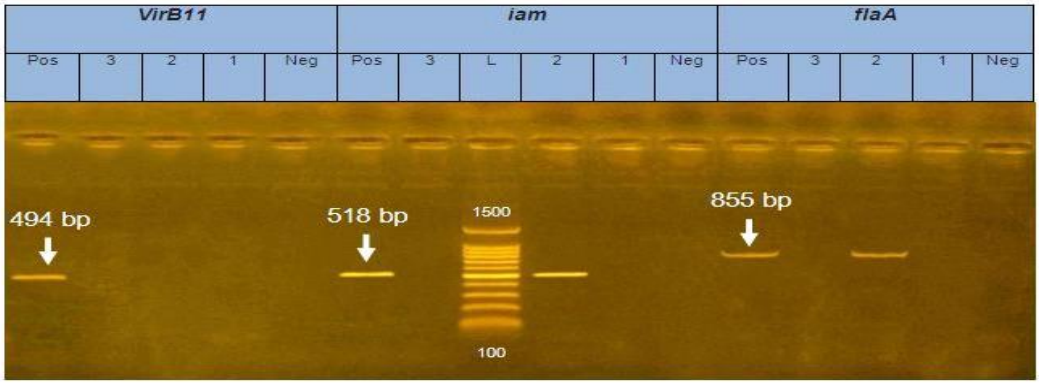

Figure (2): Agarose gel electrophoresis of specific dose-dependent amplification of *C. jejuni* pathogenic genes *(VirB11,Iam, flaA).*

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.

Discussion

Chicken are susceptible to many bacterial diseases which may be caused by a lack of hygiene, extensive exposure to the microbial contaminants and being carriers of these diseases due to their genetic immunity. Many pathogens contaminated chicken have potential risk for consumers directly from eating contaminated food by the microorganisms or through their enterotoxins. *C. jejuni* are associated in a wide range of food sources such as poultry. Symptoms of food poisoning are ranged from mild to severe include stomach pain, abdominal cramps, nausea and vomiting, diarrhea, fever, and dehydration (Abd El-Malek *et. al.*, 2010).

Egyptians have been costumed to buy live chicken from public live bird markets and slaughter it immediately after selection in the markets using the same primitive manual equipment's in slaughtering that considered excellent sources to spreading microbial contamination to the slaughtered bird. That 'on-the-spot' slaughter, plucking and evisceration in the live bird markets lead to carcass contamination and poses hazards of food borne diseases to the consumers (Khalafalla *et. al.*, 2015 and Gwida & Elgohary 2015). Meat handlers could be sources of *C. jejuni* contamination particularly in case of following improper personal hygienic precautions. Moreover, shutting down live poultry markets is extremely effective in preventing human cases.

Egyptian Organization Specification (EOS, 2005) and European regulations, (European Commission, 2005) prohibit consumption of any food containing any amount of *C. jejuni*. In the present study, the detection of *C. jejuni* with zoonotic potentials that exceed the standards indicate public health hazards to the consumers and raise the needs for proper implementation of preventative programs and regular surveillance.

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, it was illustrated that 100% of chickens infected asymptomatically by *C. jejuni* in their intestinal tracts and shading the organism through feces (Wagenaar *et al.* 2013). In this study, the total prevalence of *C. jejuni* among chicken meat and liver was 24.67%

which was consistent with other studies that recorded 22.6% in Egypt (Khalafalla et. al., 2015) and 25% in England (Jørgensen et al. 2002).

Moreover, a higher, this higher percentage could be attributed to the sampling of stool samples from human in contact with food animals (Hassanain, 2011).

However, lower C. jejuni isolation rate of 16.7% was reported in chicken meat of Giza, Egypt by (Hassanien et, al., 2011), 21.5% in Bin-suef, Egypt chickens meat by (El Fadaly, et. al., 2016). Other studies reported a very high prevalence of C. jejuni reached 40.4 % and 37.5 % in chicken liver and meat in Egypt (Barakat et. al., 2015), 51.06% in Malaysian (Ilida and Faridah, 2012), 60% in Portugal (Antunes, et. al., 2003) and even over 90% (Rozynek et al. 2008 and Tang et al. 2009). In 2008, an extensive survey on the prevalence of Campylobacter spp. in broiler carcasses from slaughterhouses in the European Union showed that 75.8% of samples were contaminated with these bacteria with 100% records in some countries (Anonymous 2010). The differences in the prevalence of C. jejuni could be related to differences in geographical area and population. This study was conducted in a desert area and very hot and dry weather might be a determinant of the prevalence rate.

Regarding the site of contamination of the examined bacteria, pectoral muscle the total contamination rate of C. jejuni was highest in liver samples 26 (26%), followed by breast muscles 25 (25%) and thigh muscles 23 (23%). Ilida and Faridah, (2012) reported that poultry samples contaminated by C. jejuni were (66.67%) of breast & liver and (75%) of thigh. These results concluded that all poultry meat are subjected to contamination by C. jejuni from intestinal contents that might be occur during improper slaughtering, buckling and evisceration processes. Compared with the numbers found on poultry meat and liver surfaces, bacterial numbers inside tissues are low but nonetheless may be significant when undercooking occurs (Khalafalla et. al., 2015).

Regarding human samples, the prevalence rate of C. jejuni among diarrheic human samples was 28%. C. jejuni was detected in 35% children stool in 3 governorates of Egypt (Barakat et al., 2015). Campylobacter organism is one of the most common causes of human bacterial gastroenteritis. About 15 of every 100,000 people are diagnosed with Campylobacteriosis every year, and with many cases going unreported, up to 0.5% of the general population may unknowingly harbor Campylobacter in their gut. All this emphasizes the importance of chickens as a potential reservoir and source of C. jejuni infection in human.

Some potential genetic markers of bacterial virulence have been identified. Fla A gene involved in adhesion and colonization, virB11gene 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 (Sanad et al., 2011; Nuijten et al., 2000; Young et al. 2007 and Dasti et al. 2010). In this study, the prevalence of VirB11 gene, fla A, and iam genes was 2.27%, 3.34%, and 26.66%, respectively in chicken meat and liver. Results of VirB11 gene in chicken was lower 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 fla A gene was detected in 3.34% of chicken meat, which was much lower than previous reports, which reached even 100% Wiseczorek et al., (2012). The iam gene was detected in 26.66% of chicken meat 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 virulence markers genes in the human isolates showed that the prevalence of VirB11 gene, fla A, and iam genes was 14.29 %, 71.43%, and 35.71%, respectively. Results of VirB11 gene was lower than that reported by Datta et al., (2003) who recorded 10·7%. Another study showed that the virB11 gene was detected in 3.6% isolates from human (Gonzalez-Hein et al, 2013). The fla A was detected in a high percentage of the isolates tested (71.34%). These results were much lower than the data previously reported by other authors (Datta et al. 2003 and Rozynek et al. 2005). The iam gene of Campylobacter was another virulence marker detected in this study. Differences in the prevalence of the iam factor were found by other

authors (Korsak *et al.* 2004; Rozynek *et al.* 2005 and Sanad *et al.*, 2011). The *iam* gene has been detected in the majority of invasive *C. jejuni* retrieved from humans. Furthermore, the detection of *iam* in *C. jejuni* isolated from two important hosts, humans and chickens, suggested a role for this marker in *C. jejuni*'s colonization of multiple hosts. 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 in humans (Korsak *et al.* 2004 and Rozynek *et al.* 2005). The detection of high rates of virulence genes among chicken and human samples referred to the potential of zoonotic infection of human by eating undercooked or improperly cooked chicken meat and liver.

Conclusion

In conclusion, this survey revealed that raw poultry meat available for consumers in Egypt was often contaminated with zoonotic *C. jejuni* that might represent a threat to public health. Furthermore, several strains were positive for the several putative virulence marker genes. The consumption of undercooked meat cross-contaminated with *C. jejuni* may pose a serious threat to consumer health. Therefore, adequate cooking of chicken meat, the personal and equipment cleanliness and chemical disinfectants, hygienic handling, storage and effectively processing of chicken meat are warranted.

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