SVU- International Journal of Veterinary Sciences, 5(4): 15-32, 2022
Print ISSN: 2535-1826 **Online ISSN: 2535-1877**

Research Article Open Access

Prevalence and Virulence Genes Profile of Zoonotic *Campylobacter* species in Chickens and Human in Aswan Governorate

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Abstract

Campylobacteriosis is considered as one of the most common zoonotic gastrointestinal diseases in the world. Because of the substantial contamination of poultry carcasses and organs with stomach contents during mechanical evisceration, birds and their products are the most common sources of *Campylobacter*. This study evaluated the mutual function of chickens in the transmission of *Campylobacter jejuni* and *Campylobacter coli* to hospitalized patients in Aswan Governorate, Egypt. Samples were collected randomly from fresh chickens (no= 108) and frozen chickens (no= 100) from different supermarkets in Aswan Province, Egypt as well as 60 diarrheal samples were assembled from hospitalized patients. Biochemical and molecular techniques were employed through duplex polymerase chain reaction objecting the *23S rRNA*, *mapA and ceuE* genes specific to genus *Campylobacter*, *C. jejuni* and *C. Coli*, respectively, after that virulence genes (*flaA* and *cadF* genes) were detected. By using conventional and duplex PCR methods, the overall incidence of *Campylobacter* was 29% and 25.4 %, respectively. *C. jejuni* and *C. coli* by conventional and PCR approaches were identified as 18.1, 5.1%, and 12.3, 7.2%, respectively, while 5.8% mixed infection was discovered by both techniques. *Campylobacter* species could be isolated from 66.7, 25, 17.5 and 18.3% of fresh chickens, frozen chickens, frozen liver and gizzard, and human, respectively with statistically significant difference. Epidemiologically, the insignificant age risk factor was statistically reported in this study among patients although *Campylobacter* was dominant in the 21-35 and 36-50 age groups. *Campylobacter* incidence was higher among females (33.3%) than in males (11.9%). On the other hand, *flaA* virulence gene was detected in 10.3% of both *C. jejuni* and *C. coli* isolated from chickens but could not be detected in human isolates. Whereas *cadF* virulence gene could be isolated in 20.5, 23.1, 36.4, and 9.1% of *C. jejuni* and *C. coli* of chickens′ and human isolates, respectively. In conclusion, the high incidence of *Campylobacter* in fresh chickens is considered the main risk factor for domestically acquired campylobacteriosis in Aswan Governorate, Egypt, confirming the urgent need for food safety strategies and emphasizing the importance of refrigeration and freezing in controlling bacterial growth in foods.

Keywords: *Campylobacter*, Fresh chickens, Frozen chickens, Human, Virulence genes.

DOI: 10.21608/svu.2022.143454.1204 Received: June 18, 2022 Accepted: August 15, 2022 Published: December 1, 2022

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Citation: Abbas et al., Prevalence and Virulence Genes Profile of Zoonotic *Campylobacter* species in Chickens and Human in Aswan Governorate. SVU-IJVS 2022, 5(4): 15-32.

Competing interest: The authors have declared that no competing interest exists.

Introduction

Campylobacteriosis is a zoonotic illness which caused by *Campylobacter* species; the organisms reported as major food-borne pathogens and the most prevalent bacterial causes of gastroenteritis in humans around the world (WHO, 2020). *Campylobacters* are Gram-negative bacilli, microaerophilic, spiral or slightly curved, motile, and non-spore forming bacteria (Salehi et al., 2014). The genus *Campylobacter* has drastically increased; and currently contains 39 species in total, including 16 subspecies. (Hlashwayo et al., 2020) from which *Campylobacter jejuni* and *Campylobacter* coli are commonly responsible for gastroenteritis in humans (Facciolà et al., 2017).

From a socioeconomic standpoint, the high incidence of *Campylobacter* diarrhea, as well as its length and its sequelae, makes it extremely essential, especially in developing and industrialized countries (Mughal, 2018; WHO, 2020). Many animal species, including poultry, cattle, pig, sheep, and goats, have been identified as natural reservoir hosts or carriers of *Campylobacter* (Skarp et al., 2016). Whereas the chicken industry is a key reservoir for *Campylobacter* species and a major cause of human transmission (Kaakoush et al., 2015).

Chickens' intestinal tracts are colonized by *Campylobacter jejuni* and *C. coli*, possibly as a result of their higher body temperature (42°C), which contributes to ideal conditions for *Campylobacter* growth in the cecum and colon, which can spread via faecal contamination at farms or during processing (Abd El-Aziz and Abd-Allah, 2017). At the abattoir, if intestinal contents spill and come into contact with faeces or contaminated equipment, carcasses can be contaminated (Facciola et al., 2017). Recent studies reported that flies may play an important role in the transmission of *Campylobacter* species to broiler chickens from contaminated sources (Royden et al., 2016).

Human campylobacteriosis mainly occurs through oral route, according to the European Food Safety Authority. Poultry meat handling, preparation, and consumption account for 20-30% of human cases of *Campylobacter* infection, with 50-80% attributable to the chickens (European Food Safety Authority, 2010). Consequently, handling and eating raw or undercooked poultry meat, in addition to cross-contamination of raw and ready-to-eat foods is a major source of human infection (Adzitey et al., 2011). In humans, the infection is usually acute and self-limiting, with diarrhea, fever, and abdominal cramps that go away within a few days to two weeks (Kim et al., 2018). However, severe illness and death can occur in young children, the elderly, and those with a compromised immune system in some cases (Same and Tamma, 2018). Infection with *Campylobacter* can cause serious complications like immune-mediated reactive arthritis (7,000 cases per 100,000) and Guillain-Barré syndrome (30 cases per 100,000) (Ford et al., 2014; American Public Health Association, 2015).

The putative virulence factors for epithelial cell adhesion and invasion, toxin production, and flagellar motility are thought to influence virulence and pathogenic mechanisms by which *Campylobacter* species cause infection (Younis et al., 2018). *Campylobacter* adhesion to fibronectin F (*CadF*) and fibronectin-like protein A are two outer membrane-embedded FNBPs found in *C. jejuni* and *C. coli* (*FlpA*). The *cadF* gene, which is involved in the invasion process, affects the microfilament organization in host cells. (Monteville et al.,2003; Bolton, 2015).

Furthermore, the extracellular filament structural components of *Campylobacter's*

bipolar flagella are made up of a hook–basal body complex containing various proteins, as well as a major flagellin protein, *FlaA* (coded by *flaA*), and a minor flagellin protein, *FlaB* (coded by *flaB*). The *flaA* gene appears to be highly conserved among *Campylobacter* isolates, and it has a higher transcription level than *flaB* (Hermans, 2011, Bolton, 2015). Thus, flagella are required for attachment to intestinal epithelial cells and are involved in motility, chemotaxis, virulence protein secretion, auto-agglutination, microcolony formation, and evasion of the innate immune response, (Guerry, 2007).

Therefore, this study aimed to assess the incidence of *Campylobacter* species in fresh and frozen chickens as well as hospitalized patients in Aswan Province, Egypt through conventional and molecular techniques, as well as, the virulence genes profile of the recovered isolates.

Materials and methods

Study design and sampling

The study was conducted during the period between 2018 and 2021 during which two hundred and eight samples were collected from fresh and frozen chickens (108 and 100 samples from fresh and frozen chickens, respectively). From fresh chickens; breast, thigh, wing, liver, gizzard and cloaca (18 samples for each) were gathered, while in frozen carcasses, samples were collected from breast, thigh, and wings, beside frozen liver and gizzard (20 samples for each). The samples were collected randomly from various markets in Aswan Province, Egypt, then transferred immediately to the laboratory for further processing in sterile and sealed containers. Twenty-five grams of each sample were added to 225mL of a selective pre-enrichment medium (Thioglycollate broth, Oxoid) and thoroughly mixed with a homogenizer before being incubated at 42 °C for 48 hours in a microaerophilic atmosphere (*Campylobacter* gas generating kits, Oxoid, BR056A, England).

On the other hand, the human survey was conducted on 60 diarrhea samples which were collected from patients admitted to medical labs and hospitals in Aswan with gastrointestinal disturbances and diarrhea in clean cups and transferred immediately to the laboratory in ice box for further examination. Age and sex of each patient were recorded. Then the samples were transferred to sterile test tubes contained Thioglycollate broth (Oxoid) and incubated in a microaerophilic atmosphere (*Campylobacter* gas generating kits, Oxoid, BR056A, England) at 42°C for 48 hours.

Isolation and identification of *Campylobacter* species:

The isolation of *Campylobacter* species followed ISO 10272-1 (ISO, 2006). A loopful of Pre-inoculated thioglycollatebroth was streaked onto modified *Campylobacter* blood free selective medium (modified charcoal-cefoperazone-deoxycholate agar (mCCDA) (Oxoid, CM0739B, England) with selective supplement (Oxoid, SR0155E, England). All inoculated plates were incubated in anaerobic jar with *Campylobacter* gas generating kits; CO_2 (10%), O_2 (5%) and nitrogen (85%) (Oxoid, BR056A, England) at 42°C for 48 hours. After that typical colonies were plated onto Columbia Blood Agar (ASC; Biolife, Milan, Italy) and incubated in a microaerophilic environment for 48 hours at 42°C. *Campylobacter* cultures were examined under a microscope to determine the isolates' morphology and motility under phase contrast microscope to demonstrate the corkscrew like motion characteristic of *Campylobacter* species. Then purified colonies were identified biochemically by catalase production, oxidase and hippurate hydrolysis tests (Roberts and Greenwood, 2002).

Molecular Identification:

DNA extraction

Following the manufacturer's instructions, genomic DNA was extracted from *Campylobacter* cultures using the QIAamp DNA Mini kit (Qiagen, GmbH, Germany). The extracted DNA was kept at -20°C until it was needed.

PCR amplification

The primers used in this study which were obtained from Metabion (Germany) and are listed in Table 1. Primers were used in a 25-µl reaction containing 12.5 µl of Emerald Amp GT PCR mastermix (2x premix) (Takara, Code No. RR310A kit), 1 µl of each primer of 20 pmol concentration, 5.5 µl of grade water, and 5 µl of DNA template. Agarose gel 1.5% was prepared in 100 ml TBE buffer. Twenty µl of each uniplex PCR product and 30 µl of each duplex PCR product, negative control and positive control were loaded to the gel. The power supply was 1-5 volts/cmGene ruler 100 bp DNA ladder (cat. no. SM0243) supplied from Fermentas**.** Finally, the gel was photographed using a gel documentation system (Alpha Innotech, Biometra, Germany) and the data were analyzed through computer software.

Statistical analysis:

Data were statistically analyzed using a SPSS version 22, and all significant levels were considered at $P < 0.05$. The association between the positive *Campylobacteriosis* and sources of the examined samples was calculated by Pearson Chi-square, Monte Carlo test or Fisher's exact test. Finally, to calculate the risk factors we used odd ratio 95.0% C.I for dichotomous variable 2X2 and binary logistic regression for multiple category variable.

Table 1. Primer sequences of *Campylobacter* genes:

Target gene	Primer sequence	Amplification (35 cycles)					bp	Reference
		Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension		
23S rRNA	TATACCGGTAAGG AGTGCTGGAG ATCAATTAACCTTC GAGCACCG	94˚C 5 min.	94˚C 30 sec	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min	650	Wang *et al*., 2002
ceuE	AATTGAAAATTGCT CCAACTATG TGATTTTATTATTT GTAGCAGCG	94˚C 5 min.	94˚C 30 sec	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min	462	Eunju and Lee, 2009
mapA	CTATTTTATTTTTG AGTGCTTGTG GCTTTATTTGCCAT TTGTTTTATTA	94˚C 5 min.	94˚C 30 sec	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min	589	
flaA	TCCAAATCGGCGC AAGTTCA TCAGCCAAAGCTC CAAGTCC	94˚C 5 min.	94˚C 30 sec	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min	217	Zheng *et al*., 2006
cadF	TTG AAG GTA ATT TAG ATA TG CTA ATA CCT AAA GTT GAA AC	94˚C 5 min.	94˚C 30 sec	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min	400	Al Amri*et al*., 2007

Results

Incidence of *Campylobacter* species in chickens and human

Data illustrated in Table 2 clarified that the overall incidence of *Campylobacter* in our study was 29% (40/138) by conventional method while was 25.4% (35/138) by using mPCR. In detail, *C. jejuni* and *C. coli* were detected in percentages of 18.1, 5.1 and 12.3, 7.2% by conventional and PCR techniques, respectively at the time mixed infection was detected in 5.8% of the screened samples. Statistically significant differences were observed between detection of *Campylobacter* and sources of the examined samples either by using conventional technique (*P*= 0.002) or by PCR (*P*= 0.000).

Fresh chickens harbor *Campylobacter* species as follows: 16.7% *C. jejuni*, 11.1% *C. coli*, and 38.9 % mixed infection. While in frozen whole chicken carcasses 5% *C. jejuni* and 20% *C. coli* could be detected. From frozen liver and gizzard, *C. jejuni* was recovered in a percentage of 15 at the time *C. coli* couldn't be detected alone, while mixed infection was obtained from 2.5% of the examined samples with statistically significant difference (*P* =0.000) as outlined in Table 3.

Data recorded in Table 4 elucidated the isolation pattern of *C. jejuni* in the examined samples that many carcasses showed the presence of the organism in more than one site of the same carcass. *C. jejuni* could be isolated from the thigh and cloaca of the same chicken (No.3), from breast and thigh of chickens (No.6 & No.12), breast and cloaca of chickens (No. 11 & No.14), and from breast and gizzard of chicken (No.9). while in frozen carcasses, *C. jejuni* was detected in wings only. On the other hand, the isolation pattern of *C. coli* was illustrated in Table 5 where it could be recovered from wing and liver of fresh carcass No. 8 and in the fresh chicken No. 10 it could be detected in the thigh and gizzard.

Table 2. Occurrence of *Campylobacter* in the examined samples using conventional method and PCR:

Ex. Chicken samples		Conventional methods								X^2 (P)	Duplex PCR								X^2 (P)
		Campylo-bacter Spp.		*C. jejuni*		*C. coli*		Mixed infection			*Campylo-bacter Spp.*		*C. jejuni*		*C. coli*		Mixed infection		
Source	No	No	%	No	%	No	%	No	%		No	%	No	%	No	%	No	%	
Fresh whole carcasses	18	12	66.7	3	16.7	2	11.1	7	38.9		12	66.7	3	16.7	2	11.1	7	38.9	
Frozen whole carcasses	20	5	25	1	5	4	20	0	0	14.603	5	25	1	5	4	20	0	0	19.096
Frozen livers and gizzard	40	8	20	7	17.5	0	0	1	2.5		7	17.5	6	15	0	0	1	2.5	
Human	60	15	25	14	23.3	1	1.7	0	0		11	18.3	7	11.7	4	6.7	0	0	
Total	138	40	29	25	18.1	7	5.1	8	5.8	(0.002)	35	25.4	17	12.3	10	7.2	8	5.8	(0.000)

X^2=Person Chi Square

Table 3. Occurrence of Campylobacter species in the examined chicken samples.

Examined chicken samples		Positive *Campylobacter* Species		*C. jejuni*		*C. coli*		Mixed infection		Monte Carlo Test.
Source	No.	No.	%	No.	%	No.	%	No.	%	
Fresh whole carcasses	18	12	66.7	3	16.7	2	11.1	7	38.9	35.621 (*P*= .000)
Frozen whole carcasses	20	5	25	1	5	4	20	0	0	
Frozen livers and gizzard	40	7	17.5	6	15	0	0	1	2.5	
Total	78	24	30.8	10	12.8	6	7.7	8	10.3	

Table 4. Isolation pattern of *C. jejuni* from the examined chickens

Sources of samples Carcass No.	Fresh chickens						Frozen chickens						
							Same carcass				Sample No.	Diff. carcasses	
	B	T	W	L	G	C	Carcass No.	B	T	W		L	G
1*	-	-	-	-	-	+	16	-	-	+	1	+	-
3*	-	+	-	-	-	+					2	-	+
6	+	+	-	-	-	-					3*	+	-
7*	+	-	-	-	-	-					4	-	+
9	+	-	-	-	+	-					5	+	-
10*	-	-	+	-	-	-					6	+	-
11*	+	-	-	-	-	+					7	+	-
12	+	+	-	-	-	-							
14*	+	-	-	-	-	+							
16*	-	-	-	-	-	+							

B=breast, **T**= thigh, **W=**wing, **L**=liver, **G**=gizzard, **C**=cloaca

Table 5. Isolation pattern of *C. coli* from the examined chickens

Sources of samples Carcass No.	Fresh chickens						Frozen chickens						
							Same carcass					Different carcasses	
	B	T	W	L	G	C	Carcass No.	B	T	W	Sample No.	L	G
1*	-	-	-	+	-	-	1	-	+	-	3*	+	-
3*	-	-	-	+	-	-	6	-	-	+			
7*	-	-	-	+	-	-	8	-	-	+			
8	-	-	+	+	-	-	10	-	-	+			
10*	-	+	-	-	+	-							
11*	-	-	-	+	-	-							
14*	-	-	-	-	+	-							
15	-	-	+	-	-	-							
16*	-	-	+	-	-	-							

Data in Table 6 showed the occurrence of *Campylobacter* species in samples obtained from fresh chickens, the highest incidence of *C.jejuni* was detected in the breast (33.3%) followed by cloaca (27.8%) and thigh (16.7%). Wings and livers showed the least incidence of *C. jejuni* (5.6%), while it couldn't be detected in the gizzard. Fresh chicken carcasses harbored *C. coli* with the acquisition of liver (27.8%) followed by wings (16.7%), gizzard (11.1%), and thigh (5.6%) while couldn't be detected in breast or cloaca.

Concerning frozen chickens, *C. jejuni* was highly detected in liver (20%), gizzard (10%), and wings (5%) while couldn't be detected in the breast and thigh. On the other hand, *C. coli* could be recovered from 15 and 5% of frozen wings and thigh, respectively, otherwise couldn't be recovered from breast, liver, and gizzard. Mixed infection was detected in liver (5%) as shown in Table 7.

Incidence of *Campylobacter* species in human

A total of 60 diarrheic patients (42 males and 18 females) aged 4-65 years were eligible to participate in this survey. The prevalence rate of *Campylobacter* in humans was 18.3% (11/60) distributed as 11.7% *C. jejuni* and 6.7% *C. coli* (Table 8). The incidence of *Campylobacter* was closely related among patients aged 21-35 years and those aged 36-50 years (26.7 and 26.3%, respectively), followed by age group of 4-20 years old (14.3%) while couldn't be recovered in the age group of 51-65 years with insignificant association (*P*=0.515) (Table 8). Also statistically insignificant difference was found between *Campylobacter* incidence in females and males (33.3 and 11.9 %, respectively) (Table 9).

A representative detection of some *Campylobacter* virulence genes were exposed in Table (10) revealing that *flaA* gene could be detected in *C. jejuni* and *C. coli* isolates obtained from chickens samples in the same percentage of 10.3, while couldn't be detected in isolates recovered from human samples. Contrarily to *cadF* gene which was detected in *C. jejuni* and *C. coli* isolates obtained from chickens and human samples as 20.5, 23.1 and 36.4, 9.1%, respectively.

Table 6. Occurrence of *Campylobacter* species in the examined fresh chickens

Sources of examined samples	No. of examined samples	+Ve*Campylobacter* species		+Ve *C. jejuni*		+Ve *C. coli*		Mixed infection		Statistical test		
		No.	%	No.	%	No.	%	No.	%	Association	Risk factor Odd ratio 95.0% C.I	
											EXP(B)	Sig
Breast	18	6	33.3	6	33.3	0	0	0	0	P= 0.000^	References	
Thigh	18	4	22.2	3	16.7	1	5.6	0	0	15.040[b]*p*=0.000	1.75(0.398-7.70)	0.459
Wing	18	4	22.2	1	5.6	3	16.7	0	0			
Liver	18	6	33.3	1	5.6	5	27.8	0	0	17.78[b]*p*=0.000	1.00(0.250-3.998)	1.000
Gizzard	18	2	11.1	0	0	2	11.1	0	0	*P*= 0.007^	4.00(0.684-23.406)	0.124
Cloaca	18	5	27.8	5	27.8	0	0	0	0	21.270[b]*p*=0.000	1.30(0.313-5.393)	0.718
Total	108	27	25	16	14.8	11	10.2	0	0			

^ = Fisher's Exact Test *b*= Monte Carlo Sig. (2-sided)

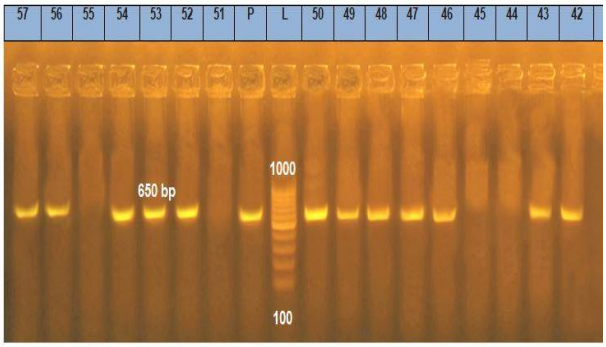

Fig. 1. PCR analysis for detection of *Campylobacter* spp. (650 bp) using *23S rRNA* gene, L: Ladder; P: positive control; N: negative control.

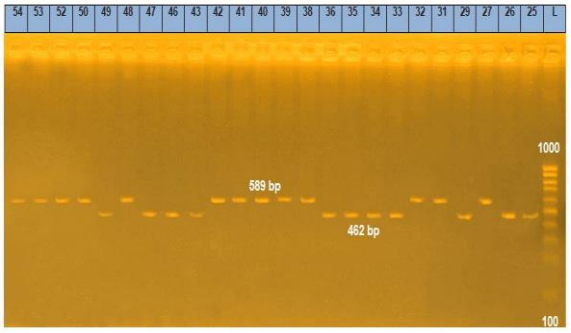

Fig. 2. duplex PCR for detection of *Campylobacter jejuni* (589 bp) using *mapA* gene and *Campylobacter coli* (462bp) using *ceuE* gene., L: Ladder; P: positive control; N: negative control

Lane 25, 26,29,33,34,35,36,43,46,47and 49: positive for *Campylobacter coli*.
Lane 27, 31,32, 38,39, 40,41,42,48, 50, 52, 53 and 54: positives for *Campylobacter jejuni.*

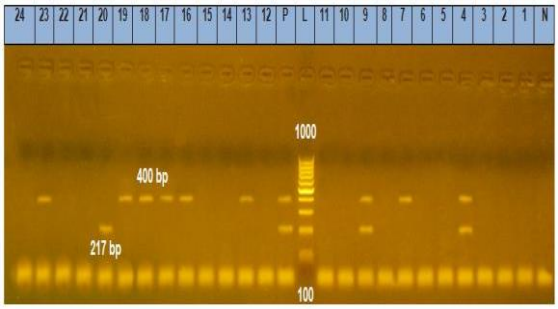

Fig. 3. duplex PCR for detection of *flaA* (217 bp) and *cadF* (400bp) virulence genes.

Table 7. Occurrence of *Campylobacter* species in the examined frozen chickens

Sources of examined samples	No. of examined samples	+Ve *campylobacter* spp		+Ve *c. Jejuni*		+Ve*c. coli*		Mixed infection		Statistical test		
		No.	%	No.	%	No.	%	No.	%	Association	Risk factor Odd ratio 95.0% C.I EXP(B)	Sig
Breast	20	0	0	0	0	0	0	0	0			
Thigh	20	1	5	0	0	1	5	0	0	P= 050^	0.333 (0.056-1.971)	0.226
Wing	20	4	20	1	5	3	15	0	0	16.085[b] p=0.000	0.444 (0.072-2.76)	0.384
Liver	20	5	25	4	20	0	0	1	5	17.871[b] p=0.000	2.111 (0.176-25.35)	0.556
Gizzard	20	2	10	2	10	0	0	0	0	P= 0.005		
Total	100	12	12	7	7	4	4	1	1			

^=Fisher's Exact Test b= Monte Carlo Sig. (2-sided)

Table 8. Occurrence of *Campylobacter* species in human samples according to the age

Age of examined humans	No. of examined samples	+Ve *Campylobacter* species		+Ve *C. jejuni*		+Ve *C. coli*		Mixed infection		Monte Carlo Test
		No.	%	No.	%	No.	%	No.	%	
4-20 years	14	2	14.3	2	14.3	0	0	0	0	5.133
21-35 years	15	4	26.7	2	13.3	2	13.3	0	0	
36-50 years	19	5	26.3	3	15.8	2	10.5	0	0	
51-65 years	12	0	0	0	0	0	0	0	0	*P*= 0 .515
Total	60	11	18.3	7	11.7	4	6.7	0	0	

Table 9. Occurrence of *Campylobacter* species in human samples according to the sex

Sex of examined humans	No. of examined samples	+Ve *Campylobacter* species		+Ve *C. jejuni*		+Ve *C. coli*		Mixed infection		Monte Carlo Test
		No.	%	No.	%	No.	%	No.	%	
Males	42	5	11.9	4	9.5	1	2.4	0	0	4.858 *P*= 0.066
Females	18	6	33.3	3	16.7	3	16.7	0	0	
Total	60	11	18.3	7	11.7	4	6.7	0	0	

Table 10. Occurrence of some virulence genes in *Campylobacter* isolates obtained from the examined sample

Sources of samples	No. of *Campylobacter* Isolates	*flaA* gene				*cadF* gene				Fisher's Exact Test
		C. jejuni		*C. coli*		*C. jejuni*		*C. coli*		
		No.	%	No.	%	No.	%	No.	%	
Chickens	39	4	10.3	4	10.3	8	20.5	9	23.1	0.287 Odds Ratio 0.680 (0.680-0.890)
Humans	11	0	0	0	0	4	36.4	1	9.1	
Total	50	4	8	4	8	12	24	10	20	

Discussion

Zoonotic *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, are major causes of food-borne bacterial infectious gastroenteritis around the world through which poultry meat products are major sources of human infections. Through our study, the overall occurrence of *campylobacter* species was (29% and 25.4%) of the examined samples by using conventional and polymerase chain reaction methods, respectively. It was observed that lower percentage of *C. jejuni* (12.3%) was detected in the examined samples by using PCR than that was detected by using conventional method (18.1%). On the contrary, *C. coli* was detected in a higher percentage (7.2%) by using PCR than that detected by using conventional method (5.1%). Identification of *C. jejuni* by conventional method depends on the result of hippurate hydrolysis test which gives a positive effect only for *C. jejuni* relies on the presence of hippuricase gene (*hip O*) (Banowary *et al*., 2015).

Campylobacter species were detected in 30.8% of examined chicken samples which were collected from different supermarkets in Aswan Governorate. This result lower than that obtained by El-Sayed *et al.* (2016) (32.3%), while higher incidence in chicken was obtained by Syarifah *et al*. (2020) (61.9%). Fresh chickens carcasses play an important role in the transmission of campylobacteriosis to human which is evident through 66.7% of the examined fresh chickens was contaminated by *Campylobacter* species versus 25% of frozen carcasses and 17.5% of the frozen liver and gizzard which may be related to many factors causing death of *Campylobacter* cells such as dehydration, ice nucleation, or oxidative stress (Maziero and de Oliveira,

2010). This obtained result was in concordance with Georgssona *et al.* (2006) who found a statistically significant reduction in *Campylobacter* isolation rate as compared with that found in fresh samples, high lighting the effect of freezing on *Campylobacter*. These results require considering public health benefits for poultry freeze before trade distribution to reduce *Campylobacter* exposure levels related to contaminated carcasses. Saiyudthong *et al.* (2015) referred to the significantly less hygienic fresh chicken meat and by-products from fresh markets than those from supermarkets.

Similar *Campylobacter* incidence in fresh chickens (66.3%) was obtained by Denis *et al.* (2001), while Maziero and de Oliveira (2010) and Food Standards Agency (2015) reported higher incidences as 93.3, and 73%, respectively. On the other hand, lower percentages (16% and 53.4%) were obtained by Lynch *et al.* (2011) and Tang et al. (2020), respectively.

Lower isolation rate from frozen carcasses were obtained by Borges *et al*. (2020) as 11.1%. Contrarily higher percentages as 36.6 and 30% were obtained by Maziero and de Oliveira (2010) and De Melo *et al*. (2021), respectively. Differences in chicken campylobacteriosis incidence rates can be attributed to a variety of factors, including the type of samples examined, location, climate factors, hygienic measures and isolation methods, as well as identification techniques. (Jorgensen *et al*., 2011 and Chatur *et al*., 2014).

Consulting to the previously obtained results, *C. jejuni* was isolated in higher rate (16.7%) than *C. coli* (11.1%) in the examined fresh chickens' carcasses and mixed infection was recorded in 38.9%. Higher percentages of *C. jejuni* (77.3% and 73.6%) were obtained by Kramer *et al.* (2000) and Reich *et al*. (2008), respectively. While Ateba *et al*. (2011) recorded a lower percentage of *C. jejuni* as 11.1 %. Nearly similar percentage of *C. coli* (10.9%) in fresh whole chickens was obtained by Jribi *et al.* (2017), however higher rate (44.4%) was obtained by Ateba *et al.* (2011), Saiyudthong*et al.* (2015) could detect lower percentage (33.3%) of mixed infection.

The opposite was occurred among frozen chicken carcasses where *C. jejuni* was isolated in the rate of 5% compared to 20% for *C. coli*. In the contrary, Maziero and de Oliveira (2010) could identify higher percentage of *C. jejuni* as 33.3%. In our study, frozen liver and gizzard samples exposed to *C. jejuni* and mixed infection in rates of 15 and 2.5%, respectively in the absence of *C. coli.* Higher percentages of *Campylobacter* species (67%) was obtained by Noormohamed and Fakhr (2012) where 34% of the frozen liver and gizzard samples were contaminated with *C. jejuni* and 33% were contaminated with *C. coli*, also Kojima *et al.* (2015) isolated *Campylobacter* from 41.2% of the examined samples. On the other hand, there is no *campylobacter* detected in frozen chicken organs by El sayed (2016). *Campylobacter* has several adaptive responses and environmental niches throughout the poultry production chain, as evidenced by the survival of this obligate microaerobic bacterium from poultry farms to slaughterhouses and final poultry products.(Hakeem and Lu,2021). Variation in the incidence of *C. jejuni* and *C. coli* in different studies might be caused by the genetic variation and resistance among thermophilic *Campylobacter* strains that explained by geographical diversity, and difference in origin of poultry-originated *Campylobacter* isolates between studies.

Concerning the isolation pattern of *C. jejuni* and *C. coli* from the same chicken carcass, they could be recovered from different sites in most of fresh chicken

carcasses especially the breast, thigh, and cloaca in case of *C. jejuni*; and wing, liver, thigh, and gizzard in case of *C. coli* which highlights the role that cross contamination plays an important role in transmitting infection. While in the frozen chicken carcasses, wing is the most common site which harbor *Campylobacter* which may be due to the traces of feathers that commonly still connected to wings that can be contaminated with feces during transportation.

In regard to the isolation rate of *Campylobacter* species from different sites of fresh chickens, a statistically significant difference (*P*= 0.000) was found between *Campylobacter* infection and different parts of the examined chickens. Breast and liver were the predominant sites of infection as 33.3% from which all isolates in breast were identified as *C. jejuni* (33.3%) while in liver the most isolate were identified as *C. coli* (27.8%). Because the birds are always hanged upside-down by their feet, leaking gut content during evisceration contaminates the lower half of the carcasses (breast and neck) more than the upper half.

The same isolation rate in breast was obtained by El-Sayed 2016, higher rate (36.6%) was detected by Jribi *et al.* (2017), while lower percentage (30.8%) was obtained by Abd El-tawab *et al*. (2015). Higher incidence of Campylobacteriosis (56.6%) in liver was recorded by De Melo et al. (2021), while Lower percentage (15.5%) was recorded by vashin *et al*. (2009). Contrarily to our result, Ali *et al.* (2016) identified most isolates obtained from liver samples as *C. jejuni* (62.2%). The higher incidence of *Campylobacter* in liver samples may be due to the intestinal rupture during evisceration and processing of the carcass which represents a threat to public health because it may lead to a great risk of pollution and infection among humans who may target the cooked chickens' liver incorrectly. In addition, the liver might be packed inside the carcasses making a good vehicle for *Campylobacter* to spread through the carcass (Stoyanchev, 2004).

The second predilection site of *Campylobacter* infection in fresh chickens was cloaca (27.8%) which represented entirely by *C. jejuni*. Higher results were obtained by Ilida and faridah (2012) and El-sayed 2016 (60% and 33.3%, respectively). In the thigh and wing 22.2% of the examined samples were infected with *Campylobacter* with the higher percentage for *C. jejuni* (16.7%) than *C. coli* (5.6%) in thighs and the opposite was in the wings. Sallam (2007) and Abd El-tawab *et al*. (2015) recorded higher percentages of *Campylobacter* species in the thigh as 70% and 38.5%, respectively. Our result in wings was lower than those obtained by Sallam (2007) and Jribi *et al.* (2017) (77.1% and28%, respectively).

The lowest occurrence rate of *Campylobacter* was in fresh chicken gizzard samples (11.1%) which represented by *C. coli* only, Incompatible result was obtained by Sallam (2007) as 45%.

Campylobacter isolation rate in frozen samples was 12 % which include 7% *C. jejuni*, 4% *C. coli* with 1% mixed infection. Nearly similar result was detected by Atterbury *et al*. (2003), El-sayed *et al.* (2016), and Borges *et al*. (2020) as 11.3, 12.5 and 11.1%, respectively. While higher percentage (36.6%) was obtained by Maziero and de Oliveira (2010). Unlike the results obtained in fresh chickens, breast of frozen chickens was found to be free from *Campylobacter* infection which was in agreement with the result obtained by Reiter *et al*. (2005). The liver was more contaminated by the organism (25%), followed by wings (20%), gizzards (10%), and finally frozen thigh had the lowest contamination rate (5%). Higher isolation rate of *Campylobacter* from frozen liver samples might be due to contamination of the organ by intestinal content during

evisceration and processing of carcasses, also the high contamination rate of frozen wings might be due to accumulation of debris containing *Campylobacter* under wings during processing and difficulty of cleaning them thoroughly. Different results were recorded by several authors as Reiter *et al*. (2005), Noormohamed and Fakhr (2012), and El-sayed (2016).

On the other hand, the overall occurrence of *Campylobacter* species in human diarrheal samples was 18.3% as identified by using PCR, with the acquisition of *C. jejuni* (11.7%). Higher incidences could be detected by siemer *et al*. (2005), Workman *et al.* (2006) and Abushahba *et al.* (2018) as 21.4, 63.6, and 27.55 %, respectively. Other authors recorded lower percentages as 15.5 and 11.4% by Quetz*et al*. (2010) and Komba *et al.* (2015), respectively. Gergs (2004) recorded nearly similar result of *C. jejuni* (12%) in human diarrhea. On the other side, *C. coli* could be identified in 6.7% of the examined diarrheal samples. Lower percentage (1.8%) was obtained by Komba *et al.* (2015).While higher results (78.6 and 31.8%) were recovered by Siemer *et al*. (2005) and Workman *et al.* (2006), respectively.

Concerning age, a high level of *Campylobacter* infection was reported at ages ranging from 21-35 years (26.7%) with equal percentages of *C. jejuni* and *C. coli* (13.3% for each).Lower result (15.4%) in the same age group was recorded by Lengerh *et al.* (2013) who isolated *C. jejuni* and *C. coli* with the percentages of 9.6% and 6.0%, respectively. Isolation rate of *Campylobacter* species in individuals aged from 36-50 years was 26.3% as the majority (15.8%) of the isolates identified as *C. jejuni.* Lower isolation rate of *C. jejuni* (12.3%) was obtained by El-Tras *et al.* (2015) and This suggested that campylobacteriosis is an endemic disease in Egypt, and the evaluated percentage of infections was for *C. jejuni*. Increasing *Campylobacter* infection rate among these two age groups, may be linked to consumption of inadequately cooked food eaten outside of the home (Kearney *et al.,* 2001).

Low *Campylobacter* infection rate was detected in the age group of 4-20 years which was 14.3% identified as *C. jejuni* only. Somewhat closely related ratios (15 and 15.4%) were obtained by Gergs (2004) and Lengerh *et al.* (2013), respectively, who found that *Campylobacter* was higher in children. On the other hand, lower percentage (0.5%) was recorded by Adekunle *et al*. (2009). Komba *et al.* (2015) recognized that young individuals were infected with higher percentage as the majority (9.6%) of the samples were *C. jejuni* and the remaining (1.8%) of the samples were *C. coli*. In our study, the older age group of 51- 65 years, has no *Campylobacter* infection as shown in Table (8).

The occurrence of *Campylobacter* species in females (33.3 %) was higher than that in males (11.9%). This result was in agreement with Youseef *et al.* (2017) who found that females possessed the higher percentage of *Campylobacter* infection (43.5%) than males (25.95%). Sexual differences are likely to play an important role in human campylobacteriosis because of the behavioral factors where women spend more time to care for young children, this may increase the risk of infection (Kuhn *et al*. 2018). Also, both estrogen and progesterone have been shown to positively affect the growth of *Campylobacter* in vitro explaining the high percentage of *Campylobacter* in women (Yokoyama *et al*. 2005 and Taylor *et al*. 2006).

Regarding to some of virulence genes which were detected in chicken and human's *Campylobacter* isolates, the occurrence of *flaA* gene which play an important role in flagellar motility was

10.3% for each *C. jejuni* and *C. coli* isolates. Higher percentage of *flaA* gene in *Campylobacter* isolates (78.5%) was obtained by Sierra-Arguello *et al.* (2021). On the other hand, none of our human *Campylobacter* isolates were positive for *flaA* gene, this result was in agreement with Kalantar *et al.* (2017), these differences might be due to the presence of multiple flagellar motility genes mutations, as these genes undergo mutations including genes deletion that occur when *Campylobacter* subjected to novel selective conditions in a non-host environment by in vitro culture in the laboratory exhibited loss of flagellar motility (Sher *et al* 2020).

Campylobacter virulence *cadF* gene (*Campylobacter* adhesin to fibronectin) play a very important role as a virulence factor in Adhesion of *Campylobacter* to fibronectin (Fn), a component ofthe extracellular matrix (Monteville *et al.* 2003). This gene was detected in chicken *Campylobacter* isolates which distributed as follows; 20.5% of *C. jejuni* and 23.1% of *C. coli*. Higher result (77.8%) was obtained by Sierra-Arguello*et al.* (2021) while Ghoneim *et al*. (2020) recorded lower result (20.58%). On the other hand, the overall occurrence of *cadF* gene in human *Campylobacter* isolates was 45.5% distributed as 36.4 and 9.1% out of *C. jejuni* and *C. coli* isolates, respectively. Association of virulence factors, *cadF and flaA* in most *C. jejuni* and *C. coli* isolates obtained from chickens' meat suggest the potential source of *Campylobacter* transmission to humans which may be harmful to the public's health.

Conclusion

This paper presented a comprehensive study of *Campylobacter* presence in chickens as a major concern for human infection. In particular, fresh chickens that are considered the main risk factor for campylobacteriosis. In addition, the presence of virulent factors in *C. jejuni* and *C. coli* isolates representing risk of *Campylobacter* infection to consumers. To reduce the risk of human exposure to *Campylobacter*, targeted control strategies are urgently needed. As a result, while hygienic measures on the farm and control measures during carcass processing can help reduce *Campylobacter* numbers, intervention at the farm level by reducing ceca colonisation should be considered as part of the overall control strategy.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethical approval

The current study was approved by Aswan University' Research and Graduate Studies Council.

Acknowledgement

The authors are grateful to all staff members of zoonosis Department, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt for their technical help.

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