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Public Health Hazard of Zoonotic *Campylobacter jejuni* Reference to Egyptian Regional and Seasonal Variations

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ABSTRACT

Several *Campylobacter* species are known to be pathogenic to humans, with *Campylobacter jejuni* being the main leading cause of campylobacteriosis worldwide. The present investigation aimed to detect *C. jejuni* from chicken, water, milk and milk products and humans among 4 Egyptian Governorates (Cairo, Fayoum, Minya and Qalubiya) using conventional method and PCR 146 *C. jejuni* isolates with an incidence of 6.2% were confirmed to species level by polymerase chain reaction through detection of *MapA* gene. high incidence of *C. jejuni* was recorded in chicken intestine (12.8%) followed by Chicken farms water (12%), raw chicken meat (9.6%), occupational human workers stool samples (8.4%) then raw milk (2%), Quraish cheese (1.7%) and finally it was 1.2% in yoghourt. The PCR was definitive, reliable method that facilitated rapid identification of *C. jejuni* to the species level. It concluded that poor hygiene and sanitation in poultry farms could explain this high level of prevalence of *C. jejuni* among the examined samples.

Key words: *Campylobacter jejuni*, poultry, milk, Egypt

INTRODUCTION

The annual number of notified campylobacteriosis cases has increased in recent years. Earlier reports suggest that the campylobacteriosis accounts for 5-15% of all human illnesses worldwide (Adak *et al*., 2005). The 2.4 million campylobacteriosis cases estimated to occur per year (Schielke *et al*., 2014). *Campylobacter* infection has been reported to occur more frequently than infections caused by *Salmonella* spp., *Shigella* spp. or *Escherichia coli* O157:H7 (CDC., 2008).

Campylobacter jejuni is a gram-negative spiral microaerophilic microorganism, which is widespread in the environment and has been detected in various animal reservoirs. Poultry has been recognized as the primary reservoir of *C. jejuni* thus, most of the campylobacteriosis infections are acquired by the consumption and/or handling of contaminated poultry meat (Malik *et al*., 2014) also consumption of unpasteurized milk, contaminated water, untreated surface water and meat rather than human to human transfer are considered as source of human infection by *Campylobacter* (Humphrey *et al*., 2007). The incubation period of *Campylobacter jejuni* microorganism typically varies from one to seven days (Butzler, 2004).

Campylobacteriosis is characterized by diarrhea, fever and abdominal cramps. The treatment is advised in old, young, pregnant and immunocompromised patients (Chatur *et al*., 2014). The most important post infectious complication of *C. jejuni* is Guillian Barre Syndrome (GBS) which

is an acute demylenating disease of peripheral nervous system characterized by paralysis of the limbs which lasts for several weeks, also include toxic megacolon, dehydration and sepsis especially in little children (<1 year of age) and immune- compromised patients (Yuki, 2001).

The present investigation aimed to detect *C. jejuni* from chicken, water, milk and milk products and humans among 4 Egyptian Governorates (Cairo, Fayoum, Minya and Qalubiya) using conventional method and PCR reflecting a view on the public health hazards.

MATERIALS AND METHODS

Sample collection: A total number of 2362 different samples were randomly collected from 4 Egyptian Governorates including Qalubiya, Cairo, Fayoum and Minya as shown in Table 1 and were examined for the presence of *Campylobacter jejuni* using isolation, biochemical and DNA characterization.

Isolation and biochemical identification of *C. jejuni* (Megraud, 1987): Loopful from each sample was cultured on thioglycolate broth and incubated at 37°C for 48-72 h. under microaerophilic condition. Primary examination of smears from the inoculated tubes were examined under phase contrast microscope using (400X) magnification power for detection of the characteristic active motility and morphology of *Campylobacter* organisms. The suspected *Campylobacter* organisms in thioglycolate broth were centrifuged at 1000-2000 rpm for 10 min. Two milliliter of the supernatant was drawn into a sterile syringe and then filtrated through 0.45-0.65 millipore filters in a swinny adaptor in a semisolid thioglycolate broth onto brucella blood agar plates with antibiotics (Skirrow Supplements). The cultured medium was incubated in gas pack system under atmosphere of 5% oxygen, 10% CO_2 and 85% nitrogen at 37 and 42°C for 48-72 h. Suspected rounded, small, translucent, grey, buffy or brownish colored colonies onto brucella blood agar plates were confirmed to be *Campylobacter* using Gram's staining and standard biochemical procedure including catalase, oxidase, hydrolysis of indoxyl acetate and hippurate hydrolysis tests (Nachamkin, 2003).

***Campylobacter jejuni* DNA recognition (El-Jakee *et al*., 2008):** A Polymerase Chain Reaction (PCR) assay targeting the *mapA* gene was used for confirmation of *C. jejuni* isolates at species level. The PCR *mapA* oligonucleotides were commercially synthesized and their sequences are as follow: The *MDmapA1* (5'CTATTTTTTTGAGTGCTTGTG3'), *DmapA2* (5'GCTTTATTTGCCATTTG TTTTATTA3'). Amplification was performed in a total reaction volume of 50 μL containing 5.0 ng DNA μL^{-1}, 40 pmol of each primer, 1 U of Taq DNA polymerase, 200 μM each dATP, dCTP, dTTP and dGTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 5.5 mM $MgCl_2$ and nuclease free water up

Table 1: Different samples collected from 4 Egyptian governorates

	Lower governorates		Upper governorates		
Samples	Qalubiya N	Cairo N	Fayoum N	Minya N	Total N
Raw chicken meat	150	120	170	240	680
Chicken intestine	63	44	37	67	211
Chicken farms water	31	23	21	33	108
Raw milk	105	127	97	128	457
Quraish cheese	81	63	77	67	288
Yoghourt	83	111	76	74	344
Human stool (occupational workers)	108	55	43	68	274
Total	621	543	521	677	2362

to 50 μL. After overlaying the mixture with mineral oil, the tubes were placed in the thermocycler and the amplification was performed under the following program: Initial denaturation at 94°C for 5 min followed by 34 cycles of denaturation at 94°C for 30 sec, annealing steps were 58°C for 30 sec and extension at 72°C for 1 min. A final extension step was done at 72°C for 10 min. The PCR products were electrophoresis at 100 V for 60 min in a 1.5% agarose gel stained with ethidium bromide (0.2-0.25 mg mL^{-1}) (Sambrook *et al*., 1989). Standard marker GeneRulerTM 10 kb DNA Ladder (Fermentus) was used. Gels were visualized under UV-lighter.

Statistical analysis: Simple one way ANOVA was used to study the effect of governorate on the incidence of *Campylobacter jejuni* using SPSS (2007). Data are presented as Means±Standard Deviation (SD). Fisher exact test was used to study the effect of season and governorate on the incidence of *Campylobacter jejuni*.

RESULTS

Incidence of *C. jejuni* among the examined samples within different governorates by isolation and biochemical identification: From the data presented in Table 2, it is clear that the incidence of *C. jejuni* within three year from 2012-2014 was highest in case of samples collected from Minya Governorate (9.6%) followed by Fayoum (7.3%), Cairo (6.1%) and finally Qalubiya (4.7%).

The incidence of *C. jejuni* within raw chicken meat samples was higher in case of samples collected from Cairo Governorate (13.3%) followed by Minya (11.3%) then Fayoum (7.6%) and finally Qalubiya (6%). The incidence of *C. jejuni* within chicken intestinal samples was higher in case of samples collected from Fayoum Governorate (16.2%) followed by Minya (13.4%) then Qalubiya (12.7%) and finally Cairo (9.1%). The incidence of *C. jejuni* within samples of Chicken farms water was higher in case of samples collected from Cairo Governorate (17.4%) followed by Minya (15.2%) then Fayoum (14.3%) and finally Qalubiya (12.9%). The incidence of *C. jejuni* within Raw milk samples was higher in case of samples collected from Minya Governorate (3.2%) followed by Fayoum (3.1%) then Qalubiya (2.9%) and finally Cairo (0.8%). The incidence of *C. jejuni* within Quraish cheese samples was higher in case of samples collected from Minya Governorate (7.5%) followed by Fayoum (3.9%) while it is not detected neither from Qalubiya nor from Cairo Governorates (0% in both of them). The incidence of *C. jejuni* within Yoghourt samples was higher in case of samples collected from Minya Governorate (8.1%) followed by Fayoum (3.9%) then

Table 2: Incidence of *Campylobacter jejuni* among the examined samples within different governorates by isolation and biochemical identification method

				Lower governorates						Upper governorates					
	Total examined samples			Qalubiya			Cairo			Fayoum			Minya		
Samples	N	+ve	%	N	+ve	%	N	+ve	%	N	+ve	%	N	+ve	%
Raw chicken meat	680	65	9.6	150	9	6.0	120	16	13.3	170	13	7.6	240	27	11.3
Chicken intestine	211	27	12.8	63	8	12.7	44	4	9.1	37	6	16.2	67	9	13.4
Chicken farms water	108	16	14.8	31	4	12.9	23	4	17.4	21	3	14.3	33	5	15.2
Raw milk	457	11	2.4	105	3	2.9	127	1	0.8	97	3	3.1	128	4	3.2
Quraish cheese	288	8	2.8	81	0	0.0	63	0	0.0	77	3	3.9	67	5	7.5
Yoghourt	344	15	4.4	83	2	2.4	111	4	3.6	76	3	3.9	74	6	8.1
Human stool (occupational workers)	274	23	8.4	108	3	2.8	55	4	7.3	43	7	16.3	68	9	13.2
Total	2362	165	7.0	621	29	4.7	543	33	6.1	521	38	7.3	677	65	9.6
Mean±SD				5.67±5.17^{ab}			4.71±5.25^{a}			5.43±3.74^{ab}			10.27±4.21^{b}		

Means with different superscripts (a, b) are significantly at b value 0.05

Cairo (3.6%) and finally Qalubiya (2.4%). The incidence of *C. jejuni* within Human stool (occupational workers) samples was higher in case of samples collected from Fayoum Governorate (16.3%) followed by Minya (13.2%) then Cairo (7.3%) and finally Qalubiya (2.8%).

The overall incidence of *C. jejuni* was higher in case of sample collected from chicken farms water (14.8%) followed by chicken intestine (12.8%) then raw chicken meat (9.6%), human stool (occupational workers) (8.4%), yoghourt (4.4%), Quraish cheese (2.8%) and finally raw milk it was 2.4%.

Governorate ($p = 0.12$) has no significant effect on mean incidence of *Campylobacter jejuni* (Table 2) but Minya Governorate has significantly high mean incidence of *Campylobacter jejuni* compared to Cairo.

Using Pearson Chi-Square, Likelihood Ratio and Fisher's Exact test to study the effect of Governorate and season on total incidence of *Campylobacter jejuni* in samples collected revealed no significance.

Confirmation of the positive *Campylobacter jejuni* isolates by polymerase chain reaction and the compatibility with isolation and biochemical identification method: The 146 *C. jejuni* isolates with an incidence of 6.2% were confirmed to species level by polymerase chain reaction through detection of *MapA* gene. high incidence of *C. jejuni* was recorded in chicken intestine (12.8%) followed by Chicken farms water (12%), raw chicken meat (9.6%), occupational human workers stool samples (8.4%) then raw milk (2%), Quraish cheese (1.7%) and finally it was 1.2% in yoghourt as shown in Table 3. *Campylobacter jejuni* isolates produced amplified fragment at the expected position 589 bp as shown in Fig. 1.

The percent of the compatibility between PCR technique and biochemical identification without PCR was 100% in case of samples of raw chicken meat, chicken intestine and occupational human workers stool. While it was 81.8% in case of raw milk, 81.3% in case of chicken farms water, while in quraish cheese it was 62.5% and finally it was 26.7% in yoghourt. The overall compatibility was 88.5% all over the study between both methods of identification.

Incidence of *Campylobacter jejuni* among the examined samples according to seasonal variations: According to seasonal variations, the highest recovery rates of *C. jejuni* tends to be obtained during summer season (no = 56) with an incidence of 8.4% followed by winter 37 (6.4%) then autumn 31 (6.2%) and finally it was 3.6% in spring (no = 22) as presented in Table 4 and Fig. 2.

Table 3: Confirmation of positive *C. jejuni* isolates by polymerase chain reaction and % of the Compatibility with Isolation and Biochemical identification method

		Results				
		Isolation and biochemical identification		Confirmed results by PCR		
Samples	Total number of samples examined	+ve	%	+ve	%	Compatibility (%)
Raw chicken meat	680	65	9.6	65	9.6	100.0
Chicken intestine	211	27	12.8	27	12.8	100.0
Chicken farms water	108	16	14.8	13	12.0	81.3
Raw milk	457	11	2.4	9	2.0	81.8
Quraish cheese	288	8	2.8	5	1.7	62.5
Yoghourt	344	15	4.4	4	1.2	26.7
Human stool (occupational workers)	274	23	8.4	23	8.4	100.0
Total	2362	165	7.0	146	6.2	88.5

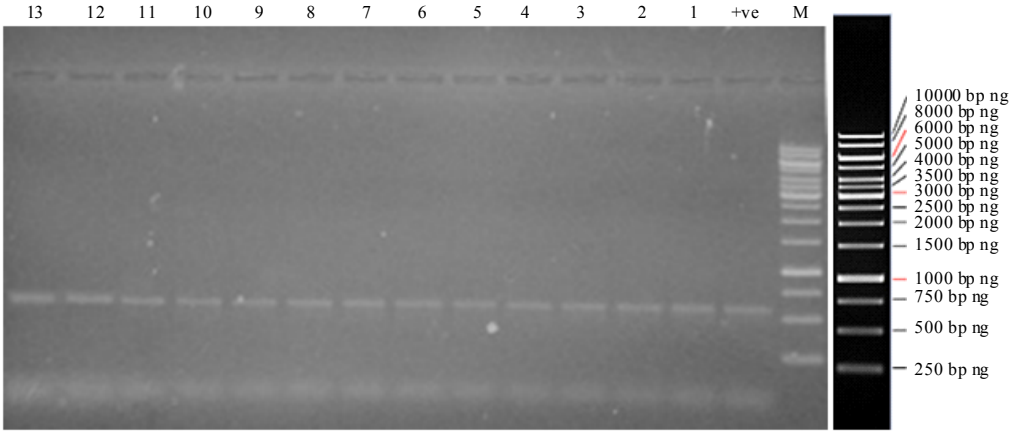

Fig. 1: Amplification of 589 bp specific to *mapA* gene for *Campylobacter jejuni*. Lane M: GeneRuler™ 10 kb DNA Ladder (Fermentus), Lane +ve: *Campylobacter jejuni* reference strain ATCC 33291, Lanes 1-13: *Campylobacter jejuni* isolates 3

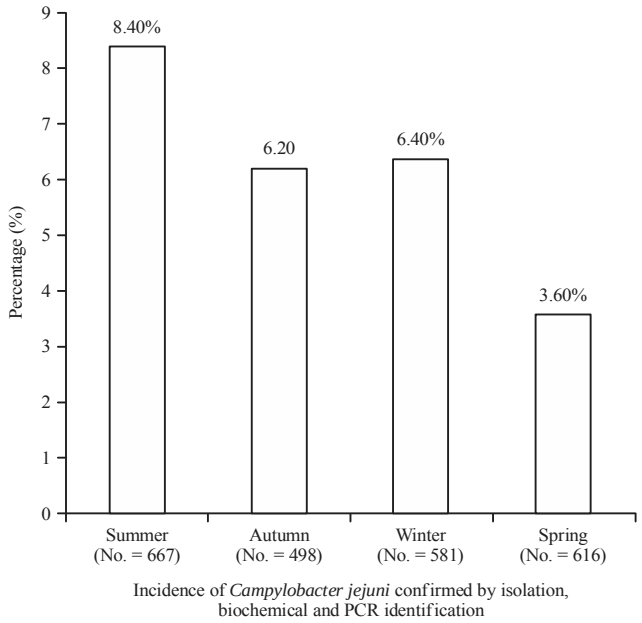

Fig. 2: Incidence of *Campylobacter jejuni* according to seasonal variation

Table 4: Incidence of *Campylobacter jejuni* according to seasonal variation within different Governorates identified by polymerase chain reaction

				Lower governorates						Upper governorates					
	Total examined samples			Qalubiya			Cairo			Fayoum			Minya		
Samples	N	+ve	%	N	+ve	%	N	+ve	%	N	+ve	%	N	+ve	%
Summer	667	56	8.4	200	14	7.0	175	12	6.9	185	10	5.4	107	20	18.7
Autumn	498	31	6.2	120	4	3.3	120	3	2.5	98	7	7.1	160	17	10.6
Winter	581	37	6.4	120	7	5.8	128	10	7.8	110	7	6.4	223	13	5.8
Spring	616	22	3.6	181	4	2.2	120	8	6.7	128	5	3.9	187	5	2.7
Total	2362	146	6.2	621	29	4.7	543	33	6.1	521	29	5.6	677	55	8.1

DISCUSSION

Campylobacteriosis is assumed to be mainly a food-borne disease (Dasti *et al*., 2010; Doorduyn *et al*., 2010; Man, 2011). In the present study from the data presented in Table 2, it is clear that the incidence of *C. jejuni* identified by traditional method within three year from 2012-2014 was highest in case of samples collected from chicken farms water (14.8%) followed by chicken intestine (12.8%) then raw chicken meat (9.6%). Newell and Fearnley (2003) pointed that contamination of the water lines usually follows rather than precedes colonization of the flock, it may be hypothesized that poor disinfection of water-line may be responsible for the *Campylobacter*-detection in the following flocks. As pointed out by Newell and Fearnley (2003), their study showed that cleaning and disinfection of water-line between flocks may help to reduce the risk of chicken *Campylobacter* colonization. Indeed, unsuitable hygiene practices at the farm level, especially poor cleaning and disinfection of the house and non dedicated protective clothing, could then be a major reason of *Campylobacter* transmission to human being (Allen and Newell, 2005). The water-line, the delivery tray and the anteroom floor swabs, whereas broilers cecal droppings were infected later, this may have been due partly to the residual presence of pathogens either from previous *Campylobacter*-positive flocks or to the environmental contamination of the house surroundings from which the infection could have arisen. *Campylobacter* species are ubiquitous in the environment and around broiler houses and may be easily transported into the human workers either in utilities, such as feed, litter and water.

Routine monitoring of zoonotic pathogens in food and farmed animals revealed that the prevalence of *Campylobacter* in poultry meat ranged from 14-34% per year (European Food Safety Authority, 2012; Federal Institute for Risk Assessment, 2012). Such a high isolation rate of thermophilic *Campylobacter* in chickens has also been reported (Cardinale *et al*., 2003; Saleha, 2004). This may partly explain the incidence of *Campylobacter* infections in our study as poultry meat is frequently contaminated with *Campylobacter*.

Case-control studies of food borne infection rates have estimated that 50-70% of *Campylobacter* illness is due to consumption of contaminated poultry and their products (Tauxe *et al*., 1985; Allos, 2001; Kapperud *et al*., 2003). Several studies examined thermophilic *Campylobacter* in poultry and the findings indicated prevalence ranges of the *Campylobacter* from 3-98% with *C. jejuni* as the main isolates (Kapperud *et al*., 2003; Cardinale *et al*., 2003). Van Looveren *et al*. (2001) found that among 677 *Campylobacter* isolates from broiler carcasses from Belgian slaughter houses, 79% was identified as *C. jejuni*.

Poor hygiene and sanitation in poultry farms could explain this high level of prevalence of *Campylobacter*. Indeed, most farms do not have security fence to prevent penetration of other animals including rats, which are good carriers of *Campylobacter*. In some cases flocks of sheep or cattle and poultry take place at the same sites contributing like that to the contamination of chicken flocks with *Campylobacter*. Furthermore, no measures of hygiene are observed in poultry farms and in the process of slaughter which could cause contamination of poultry carcasses. Since campylobacteriosis is transmitted through human activities as entrance of farmers, maintenance staff, veterinarians, visitors and catching crews and equipments that carry campylobacters.

In the present study, among 274 human (occupational workers) stool specimens examined by traditional method, 23 *C. jejuni* were isolated with an incidence of 8.4%. In another study, out of 327 human stool samples that were examined for the presence of *Campylobacter* by direct isolation on Skirrow's media and identification from the culture and biochemical reactions, 19 (5.8%) were identified as *Campylobacter*. The 17 (89.5%) out of the 19 positive isolates were identified as *C. jejuni* (Girgis *et al*., 2014).

Milk and dairy products have been previously reported as vectors in the transmission of *Campylobacter* species (Park, 2002). Consumption of raw milk, inadequately pasteurized milk and cheese contaminated with *Campylobacter* was shown to be responsible for six enteric infection outbreaks reported in England and Wales since 1981 (Dijuretic *et al*., 1997; Pebody *et al*., 1997).

From the data presented in Table 2, present study showed that the incidence of *Campylobacter jejuni* microorganisms examined by traditional method was 4.4% in yoghourt samples while in quraish cheese it was 2.8% and finally raw milk it was 2.4%.

The occurrence of *Campylobacter* species in traditional dairy products could be due to environmental contamination with infected animal wastes or unsanitary food production and storage practices (Rahimi *et al*., 2013).

Although the prevalence of *Campylobacter* spp., may vary in different dairy products, it has been shown in another study that *Campylobacter* isolates can be found more frequently in raw milk samples and soft cheeses (El-Sharoud, 2009; Hussain *et al*., 2007; Salihu *et al*., 2010). In a study in Pakestan, *Campylobacter* species were detected in 10.2 and 11.7% of raw milk and cheese samples (Hussain *et al*., 2007). In a study in Egypt, 2 of 50 raw milk samples (4.0%) and 4 of 38 fresh domiati cheese samples (11.0%) were positive for *Campylobacter* species, in which *Campylobacter* isolates recovered from these two product were all identified as *C. jejuni* (El-Sharoud, 2009).

In another study, no *Campylobacter* species was isolated from 115 pasteurized milk and commercial dairy product samples, although 13 of the 437 raw milk and traditional (3.0%) dairy product samples were positive for *Campylobacter* species (Rahimi *et al*., 2013). Although, the prevalence of *Campylobacter* species may vary in different dairy products, it has been shown that *Campylobacter* isolates can be found more frequently in raw milk samples and soft cheeses (Hussain *et al*., 2007; El-Sharoud, 2009; Salihu *et al*., 2010).

From the data presented in Table 2 and Fig. 1, it is clear that there are geographical variations in the incidence of *Campylobacter* contributing to geographical distribution within four Governorates. The incidence of *C. jejuni* within three year from 2012-2014 was highest in case of samples collected from Minya Governorate (9.6%) followed by Fayoum (7.3%) then Cairo (6.1%) and finally Qalubiya Governorate (4.7%).

The incidence of *C. jejuni* within raw chicken meat samples was higher in case of samples collected from Cairo Governorate (13.3%) followed by Minya (11.3%) then Fayoum (7.6%) and finally Qalubiya (6%). The incidence of *C. jejuni* within chicken intestinal samples was higher in case of samples collected from Fayoum Governorate (16.2%) followed by Minya (13.4%) then Qalubiya (12.7%) and finally Cairo (9.1%). The incidence of *C. jejuni* within samples of Chicken farms water was higher in case of samples collected from Cairo Governorate (17.4%) followed by Minya (15.2%) then Fayoum (14.3%) and finally Qalubiya (12.9%).

It was also observed from the milk and dairy product examination that the incidence of *Campylobacter* within unpasteurized raw milk samples was higher in case of samples collected from Minya Governorate (3.2%) followed by Fayoum (3.1%) then Qalubiya (2.9%) and finally Cairo (0.8%). The incidence of *C. jejuni* within Quraish cheese samples was higher in case of samples collected from Minya Governorate (7.5%) followed by Fayoum (3.9%) while it is not detected neither from Qalubiya nor from Cairo Governorates (0% in both of them). The incidence of *C. jejuni* within Yoghourt samples was higher in case of samples collected from Minya Governorate (8.1%) followed by Fayoum (3.9%) then Cairo (3.6%) and finally Qalubiya (2.4%). Finally, occupational human stool

samples that was taken from workers come in contact with previous collected samples, it was observed that the incidence of *C. jejuni* was higher in case of samples collected from Fayoum Governorate (16.3%) followed by Minya (13.2%) then Cairo (7.3%) and finally Qalubiya (2.8%).

It was observed that all over four Governorates, the Incidence of *Campylobacter jejuni* among the chicken intestinal samples was 260(38.2%) while it was 80 (40%) among the chicken meat samples and it was 30 (30%) among the farms water samples (from chicken farms) on the other hand it was 20 (4.5%) among the unpasteurized raw milk samples and it was 20 (7.7%) among the quraish cheese samples while among the yoghourt samples it was 40 (13.4%) and finally it was 80 (33.3%) among the Stool samples from occupational human workers.

The incidence of *Campylobacter jejuni* was high in case of samples collected from Fayoum 178(33.6%) and Minya Governorates 200(32.8%), respectively followed by Cairo Governorate 95(20.2%) then finally Qalubiya Governorate 57(9.2%).

This variations between four Governorates could be due to differences in geographical location, study population, study period (Lengerh *et al*., 2013). Furthermore differences in weather between Governorates; warm weather may have triggered recreational activities with enhanced exposure to possible risk factors, for example consumption of undercooked chicken meat (Doorduyn *et al*., 2010) or swimming in contaminated water (Dasti *et al*., 2010). In Germany, *Campylobacter* incidence peaks both in rural as well as urban areas in the summer (Schielke *et al*., 2014). In another study, the prevalence of *C. jejuni* varies in different part of Iran: Tehran, 8% (23); Semnan, 9.8% (24) and Shiraz, 9.8% because of different reasons such as level of hygiene, nutrition, weather and multi-cultural population in this city (Salehi *et al*., 2014). These data show similarity to other studies in other developing countries such as China, Bangladesh, Thailand, Egypt, Jordan, Nigeria (Coker *et al*., 2002) and Pakistan (Butzler and Skirrow, 1979) in which *C. jejuni* has been a common enteropathogen.

Hygienic measures in low socio-economic localities maximizing the common routes of transmission for campylobacteriosis by fecal-oral, person-to-person, ingestion of contaminated food and waterborne, contact with contaminated poultry, livestock or household pets, especially feces from puppies, kittens and birds (Humphrey *et al*., 2007).

A correlation between temperature and a number of campylobacteriosis cases has been described before and may also explain the seasonal pattern of the disease with an incidence peak in the summer months, which has been described for many countries (Lal *et al*., 2012; Nichols *et al*., 2012). Contamination of broilers and chicken meat with *Campylobacter* tends to be higher in the summer months; as it was found to be highest in August (Federal Institute for Risk Assessment, 2010). Chicken at retail examined during a one-year study in Germany showed two peaks of contamination, one from February to March and the second from July-August (Scherer *et al*., 2006).

A season effect for *Campylobacter* presence is generally reported in the literature (Refregier-Petton *et al*., 2001; Bouwknegt *et al*., 2004). According to seasonal variations in this study, the highest recovery rates of *C. jejuni* tends to be obtained during summer season (no = 56) with an incidence of 8.4% followed by winter 37 (6.4%) then autumn 31 (6.2%) and finally it was 3.6% in spring (no = 22) as presented in Table 4 and Fig. 2. The reason for these seasonal variations is still debated but may indicate a possible relationship between temperature and *Campylobacter* survival and transmission of infection as stated by Patrick *et al*. (2004). Insects have been frequently implicated in this seasonal effect of *Campylobacter* prevalence. Insects may be an

important source of *Campylobacter* infection. In summer, hundreds of flies passed through the ventilation system into the broiler house and the influx of insects was correlated with the outdoor temperature (Hald *et al*., 2008).

Campylobacter jejuni account for the majority of human infections. Despite considerable efforts there are still many gaps in our knowledge regarding optimal isolation and identification techniques of *C. jejuni*. Identification to species level is hindered by variations in methodology and the subjective interpretation of biochemical test results (Linton *et al*., 1997). *Campylobacter* requires special growth conditions and is not able to multiply in an aerobic atmosphere, the bacteria may survive on food or in the environment for several days (Robinson, 1981; Skirrow, 1977). Additionally, the infective dose for humans is very low (Robinson, 1981). There are also isolates with atypical phenotypes. For example, the differentiation of *C. jejuni* from *C. coli* relies on the ability of *C. jejuni* to hydrolyze hippurate (Roop *et al*., 1984), but certain atypical *C. jejuni* strains fail to do so (Roop *et al*., 1984; Nicholson and Patton, 1993), rendering identification based on this single test unreliable. These limitations might in principle be overcome by the use of PCR-based genotypic methods.

The PCR assays were used in this study for rapid and definitive identification of *C. jejuni* to the species level. Identification of *C. jejuni* on the species level is in usual relies on relatively few phenotypic tests. For example, *C. jejuni* and *C. coli* are distinguished only by hippurate hydrolysis, while *E. coli* and *C. upsaliensis* are distinguished by the weak catalase activity and sensitivity to cephalothin of the latter species. Due to these limitations, clinical laboratories often report these enteropathogens simply as *Campylobacter* species (Linton *et al*., 1997). Even when a rapid hippurate hydrolysis phenotypic test is performed to identify *C. jejuni* isolates, significant difficulty remains in the identification of any hippurate negative isolates, which could belong to other *Campylobacter* species or could indeed be hippurate-negative strains of *C. jejuni*, certain hippurate-negative strains, initially classified as *C. coli*, reacted against antisera raised against *C. jejuni*, suggesting that these strains were indeed hippurate-negative *C. jejuni* strains (Nicholson and Patton, 1993). The PCR is a method which is definitive, reliable, easy to use and are required to facilitate rapid identification of *C. jejuni* to the species level (Linton *et al*., 1997). Several PCR-based assays have been developed to facilitate the differentiation of *C. jejuni* from *C. coli*. A previous study was done by El-Jakee *et al*. (2008) to detect *C. jejuni* isolates through amplification of fragment at 589 bp specific for *MapA* gene.

In this study, *Campylobacter* isolates were confirmed to be *C. jejuni* through detection of *MapA* gene. One hundred and fifty six *C. jejuni* isolates with an incidence of 6.2% were confirmed to species level by polymerase chain reaction. high incidence of *C. jejuni* was recorded in chicken intestine (12.8%) followed by Chicken farms water (12%), raw chicken meat (9.6%), occupational human workers stool samples (8.4%) then raw milk (2%), Quraish cheese (1.7%) and finally it was 1.2% in yoghourt as shown in Table 3. *Campylobacter jejuni* isolates produced amplified fragment at the expected position 589 bp. as shown in Fig. 2.

The percent of the compatibility between PCR technique and biochemical identification without PCR was 100% in case of samples of raw chicken meat, chicken intestine and occupational human workers stool. While it was 81.8% in case of raw milk, 81.3% in case of chicken farms water, while in quraish cheese it was 62.5% and finally it was 26.7% in yoghourt. The overall compatibility was 88.5 % all over the study between both methods of identification.

CONCLUSION

The present study indicates that infection caused by *Campylobacter* species was very frequent among the occupational human workers. Efforts should be undertaken to control campylobacteriosis and to reduce infection risks. Prevention measures should include strengthening efforts to reduce *Campylobacter* prevalence in farm animals and food as suitable biosecurity measures to exclude *Campylobacter*. The development of supplementary on-farm control strategies may be required to achieve predominantly *Campylobacter* negative flocks which in turn will prevent campylobacteriosis. However, the carrying of *Campylobacter* into the human has yet to be proven by genotyping confirmation of strains in the environment which subsequently result in human colonization.

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