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MOLECULAR CHARACTERIZATION OF *CAMPYLOBACTER* SPECIES FROM TURKEYS FLOCKS IN DELTA GOVERNMENTS

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ABSTRACT

Two hundred and forty samples were collected from turkey farms and various markets in Delta Governorates for *Campylobacter* spp. isolation. Multiplex PCR assay was used to identify 2 Thermophilic *Campylobacter* species (*C. jejuni* and *C. coli*). The prevalence of *Campylobacter* in turkeys (22.5%) observed in this study; *C. jejuni* was 15.4% and *C. coli* 7%. The high incidence of *Campylobacter* in liver was (30%) followed by skin (26.7%), cloacal swabs (21.3%) and finally intestinal content (16.7%). A food borne disease infected with campylobacteriosis resulting from consuming or handling of contaminated poultry meat. The high incidence of *Campylobacter* due to contamination to intestinal tract during evisceration can lead to direct contamination of the carcasses or indirectly through the hands of the processors, material or instrument used in processing. The amplification of the DNA belonging to *Campylobacter* genus specific (*C. jejuni* hipO) was (19%); (13.8%) *C. jejuni* at 323 bp and (5.8%) *C. coli* at 126 bp, while for *C. jejuni* 23S rRNA at 650 bp. PCR analysis for detection of *Campylobacter* can be utilized as a simple, rapid and sensitive tool to discriminate stains recovered from different sources, especially when used as profile analysis for a control strategy of *C. jejuni* in turkey farms to reduce the risk of human exposure to *Campylobacter* and is an important food safety issue. Multiplex PCR was found to be more reliable than the conventional cultural methods in species level for identification of *Campylobacter* isolates. Further epidemiological studies at different geographical areas, carried to discover the prevalence, magnitude and importance of *Campylobacter* infection in turkey farms.

Keywords: *Campylobacter*, Turkey, multiplex PCR.

INTRODUCTION

Campylobacteriosis is considered as the major important zoonotic gastrointestinal disease around the world caused mainly by *C. jejuni* (Gormley *et al.*, 2008). *Campylobacter* species mainly *C. jejuni* and *C. coli* cause acute human gastroenteritis (Friedman *et al.*, 2000). *C. jejuni* is part of normal intestinal flora of poultry and is present within the first few days post-hatch. The avian intestine seems to be a favourable environment for proliferation of *C. jejuni* (Lee and Newell 2006).

Campylobacter species are recognized as the most common cause of foodborne bacterial gastroenteritis in human (Nguyen *et al*., 2016). Campylobacteriosis are predominantly caused by the Thermophilic C*ampylobacter* including *C. jejuni* and *C. coli* (Griffiths and Park, 1990). The main route of infection is ingestion of food of animal origin and particularly consumption of poultry meat (Butzler and Oosterom 1991).

Campylobacter has been found to contaminate 81% of fresh, whole broiler chicken carcasses and a third of turkey carcasses (Anonymous, 2007). *Campylobacter* is present in the crop and ceca contents; thus, a single rupture early in processing can contaminate carcasses from several flocks (Musgrove *et al*., 2001).

Thermophilic *Campylobacter* species have Gram negative cell wall with capsule and flagella. The bacteria are slender, curved rod to small spiral shape with 0.2-0.5mm width and 0.5-5.0 mm length. They need microaerophilic atmosphere at 37-42°C for 48 ±

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4 hours for optimal growth (Shane and Harrington, 1998).

Campylobacter can colonize normally in the intestine, so it can directly contaminate the meat product during evisceration inside the slaughter houses and is the major source of transmission of disease to human (Rahimi and Tajbakhsh, 2008). Meat of broilers, turkeys, and ducks are major sources of *C. jejuni* for humans (Friesema *et al.,* 2012). Turkey products are an important commercial poultry commodity and limited information is available on colonization of turkeys with *Campylobacter.*

Campylobacters increased from day l to day 14 in the carriage rate and diarrhea. By day 39, the excretion rate had reached 6 x 10 (7) campylobacters in fresh faeces, peaks in Campylobacter numbers on days 19 and 75, corresponding to peaks in diarrheic samples (Wallace *et al.,* 1998). The introduction of new birds resulted in an increase in *Campylobacter* in birds with diarrhea. Turkeys at slaughter showed increased of *Campylobacter* from beak and highest in caeca (Zhao *et al.,* 2001). Up to 34.9% of examined turkey carcasses were positive for *Campylobacter* after chilling (Logue *et al.,* 2003).

Birds infected with *Campylobacter* will contaminate the food processing environment. The concentration of *Campylobacter* on turkey carcasses and in caeca is positively correlated to human campylobacteriosis cases (Rosenquist *et al.,* 2003 and Anonymous, 2007). *Campylobacter* will have a positive impact on consumers' perceptions related to food safety, the food industry and public health agencies (Wassenar *et al*., 2007).

When considering the overall burden of campylobacteriosis, in addition to the corresponding acute morbidity and mortality described above as well as the long-term post-infectious squealed a consideration must also be made regarding anti-microbial resistance (AMR) (Poly *et al*., 2019*).*

In USA, over two million cases of *Campylobacter* related illness are reported annually. *Campylobacter* infections being responsible for 5% of food-related deaths (Mead *et al.,* 1999). In 2004, the incidence of *Campylobacter* infection in the European Union exceeded than of *Salmonella* infection for the first time (Zoonotic Agents, 2005). Chicken meat has been reported to be contaminated with *Campylobacter*s up to 100% the major sources of infection of *C. jejuni* (Borck and Pedersen, 2005).

Turkey meat is increasingly being chosen by consumers because the adherence to low-fat diets. The consumption of turkey meat has increased in recent times. It is of great importance to identify and assess the potential risks arising from turkey products. The aim of this study was to examine turkey flocks and turkey meat retail products in Delta Governorates for the prevalence of *Campylobacter* by convential cultural method and molecular characterization.

MATERIALS AND METHODS

1- *Sampling:*

Two hundred and forty samples were collected from turkeys (150 cloacal swabs from turkey farms and 90 samples from freshly slaughtered turkeys from various markets) in Delta Governorates from May 2018 till June 2019 for *Campylobacter* spp. isolation (Table, I). The samples were cultured onto thioglycollate media within four hours (Smibert, 1974).

Table I: Number and types of examined samples of turkeys for *Campylobacter* Spp.

Delta Governorates	No. of examined samples	Turkey Farms	Freshly slaughtered turkey		
		No. of Cloacal Samples	Intestinal content	Liver	skin
Sharkia	50	35	5	5	5
Dakahlia	75	45	10	10	10
Gharbia	65	35	10	10	10
Kafr El-Shaikh	50	35	5	5	5
Total	**240**	**150**	**30**	**30**	**30**

2- Isolation and identification of Campylobacter isolates:

A loop full from each sample were cultured directly onto thioglycollate medium for 24-72 hours in sterile tubes, then a loop full from each tube was cultured on modified *Campylobacter* blood free selective medium with antibiotics and 10% sheep blood. All inoculated plates were incubated in microaerophilic condition contain CO_2 (10%), O_2 (5%) and N_2 (85%) in 37ºC for 48 hours and were demonstrated for characteristics colonies. The suspected colonies will be identified by biochemical test described by El-Gohary (1998).

3- Multiplex PCR assay:

Extraction of bacterial DNA from *Campylobacter* species was performed in a 0.5 ml Eppendorf tube containing PBS by heating at 100°C for 10 min in a heat block according to OIE (2008). After centrifugation DNA was collected and stored till used. Quantification of genomic DNA extracted from *Campylobacter* isolates by using UV Visible Spectrophotometer.

Oligonucleotides primers used were supplied from Metabion (Germany) are listed in Table (2), according to Wang *et al.* (2002).

DNA amplification of Campylobacter: The amplification of DNA in a 50 µl reaction containing thermoscientific Taq for Multiplex PCR Master Mix (2X) was carried out in Bio-RAD thermo cycler with positive and negative controls and cycling condition was illustrated in Table (3).

Detection of PCR products using agarose gel electrophoresis:
The amplified PCR products were electrophosed on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer with ethidium bromide. For gel analysis, 10 µl of the products was loaded in each gel slot. Thermoscientific, Gene Ruler 100 bp ladder was used to determine the expected fragment sizes (Sambrook *et al*., 1989). The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

Table 2: Oligonucleotides primers used for detection of *Campylobacter* strains in Turkey by Multiplex PCR.

Primer name	Target gene	Sequence (5'–3')	Accession No.	Gene location	size (bp)	Reference
23SF	*C. jejuni* 23S rRNA	TATACCGGTAAGGAGTGCTGGAG	Z29326	3807–3829	650	Wang *et al*., 2002
23SR		ATCAATTAACCTTCGAGCACCG		4456–4435		
CJF	*C. jejuni hipO*	ACTTCTTTATTGCTTGCTGC	Z36940	1662–1681	323	
CJR		GCCACAACAAGTAAAGAAGC		1984–1965		
CCF	*C. coli* glyA	GTAAAACCAAAGCTTATCGTG	AF136494	337–357	126	
CCR		TCCAGCAATGTGTGCAATG		462–444		

Table 3: Cycling conditions of the primers during PCR according to (Wang *et al*., 2002).

Step	Temperature	Time	Number of cycles
1. Primary denaturation	94˚C	6 min.	1 cycle
2. Cycling			
A. Secondary denaturation	95˚C	30 sec.	35 cycles
B. Annealing	59˚C	30 sec.	
C. Extension	72˚C	30 sec.	
3. Final extension	72˚C	7 min.	1 cycle

RESULTS

Table 4: Detection of *C. jejuni* and *C. coli* in turkey samples by conventional cultural method.

Delta Governorates	No. of the examined samples	No. of positive samples		Conventional cultural methods			
				C. jejuni		*C. coli*	
		No.	%	No.	%	No.	%
Sharkia	50	10	20	6	12	4	8
Dakahlia	75	18	24	12	16	6	8
Gharbia	65	14	21.5	9	13.8	5	7.7
Kafr El-Shaikh	50	12	24	10	20	2	4
Total	**240**	**54**	**22.5**	**37**	**15.4**	**17**	**7**

Table 5: Occurrence of *Campylobacter jejuni* and *Campylobacter coli* in different turkey samples.

Types of examined turkey samples	No. of examined samples	Positive *Campylobacter* species		*Campylobacter jejuni*		*Campylobacter coli*	
		No.	%	No.	%	No.	%
Cloacal samples	150	32	21.3	24	16	8	5.3
Intestinal content	30	5	16.7	4	13.3	1	3.3
liver	30	9	30	5	16.7	4	13.3
skin	30	8	26.7	4	13.3	4	13.3
Total	240	54	22.5	37	15.4	17	7

Table 6: Biochemical tests to differentiate between *C. jejuni* and *C. coli.*

Characteristics	*C. jejuni*	*C. coli*
Oxidase	+	+
Catalase	+	+
Nitrate reduction	+	+
Urease	--	--
Hippurate hydrolysis	+	--
Growth at:		
37◦C	+	+
43◦C	+	+
Growth at 1% glycine	+	+
Susceptibility to:		
Nalidixic acid	**S**	**S**
Cephalothin	**R**	**R**

Table 7: Incidence of *Campylobacter jejuni* and *Campylobacter coli* by multiplex PCR.

Types of examined turkey samples	No. of examined samples	Positive *Campylobacter* spp.		*Campylobacter jejuni*		*Campylobacter coli*	
		No.	%	No.	%	No.	%
Cloacal samples	150	30	20	22	14.7	8	5.3
Intestinal content	30	3	10	2	6.7	1	3.3
liver	30	8	26.7	5	20	3	10
skin	30	6	20	4	13.3	2	6.7
Total	240	47	19.6	33	13.8	14	5.8

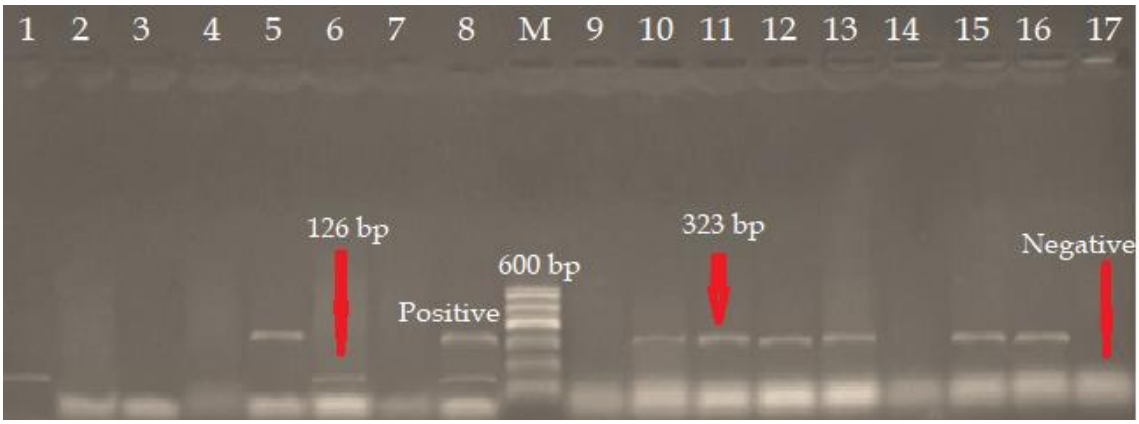

Fig. (1): Multiplex PCR of *C. jejuni hipO* and *C. coli* were detected in turkey samples in 1.5% agarose gels electrophoresis. All the 47 *Campylobacter* isolates yielded the genus specific as 31 isolates of *C. jejuni* at 323 bp while 14 produced the *C. coli* at 126 bp. Lane 1 and 6 samples positive of *C. coli*. Lane: 5,10,11,12,13,15 and 16 samples positive of *C. jejuni.* M: 100-600 bp ladders; Lane 8: Positive controls of *C. jejuni and C. coli*. Lane 7: Negative control.

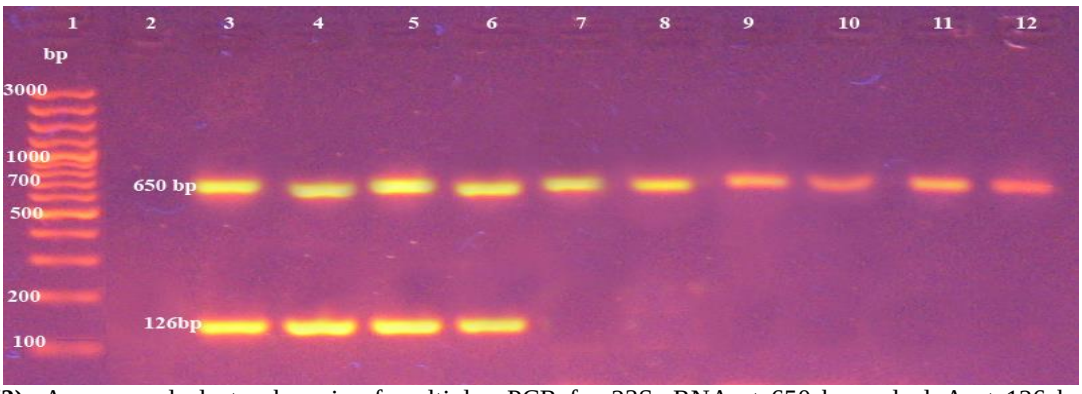

Fig. (2): Agarose gel electrophoresis of multiplex PCR for 23S rRNA at 650 bp and glyA at 126 bp from turkeys. Lane 1: Thermoscientific, GeneRuler 100bp DNA Ladder, (#SM0243). Lane 2: control Negative. Lane 3: Positive control for *C. jejuni* and *C. coli.* Lanes 4-12: samples positive *C. jejuni* for 23S rRNA in all *Campylobacter* spp. with *C. coli* at 126 bp, except 7- 12 lane has no *C. coli.*

DISCUSSION

The prevalence of Campylobacter in turkeys (22.5%) observed in this study as shown in table (4) is with values reported by Noormohamed and Fakhr (2014) and Logue *et al*. (2003). The highest incidence was in Dakahlia and Kafr El-Sheikh Governorates 24% followed by Gharbia 21.5% and finally in Sharkia 20% (Table, 4). The prevalence of *Campylobacter jejuni* in turkeys was 15.4% which is near from results obtained by Perko-Makela *et al*. (2009). *Campylobacter coli* percentage in turkeys was detected as 7% which was agreement with Atanassova *et al*. (2007) and Gahlan *et al*. (2017). The prevalence rate of *C. jejuni* was higher than *C. coli* in this study which agree with Weber *et al*. (2014). The difference was notably due to a positive hippurate test identified as *C.jejuni* but absence for *C. coli* (Table, 6) (Sincinschi, 1995).

Table (6) revealed the high incidence of *Campylobacter* in liver was (30%) followed by skin (26.7%), cloacal swabs (21.3%) and finally intestinal content (16.7%). The high incidence of *Campylobacter* due to contamination rate attributed to that damage to intestinal tract during evisceration can lead to direct contamination of the carcasses. Contamination can also occur indirectly through the hands of the processors and material or instrument used in processing (Salihu *et al*., 2009).

The Polymerase Chain Reaction (PCR) technique is the most widely used of all molecular techniques as it is highly sensitive, specific and rapid for the detection of food-borne pathogens (Samosornsuk *et al*., 2007). The specificity of this assay to detect *C. coli* or *C. jejuni* was 97% (Nayak *et al*., 2005). Table (7) showed the amplification of the DNA belonging to *Campylobacter* isolates obtained in the present study. This amplification was performed strictly according to the Multiplex PCR methods and amplification parameters as specified by Wang *et al*. (2002). All samples yielded the genus specific (23S rRNA) with percentage (19%); (13.8%) *C. jejuni* specific at 650 bp and (5.8%) *C. coli* specific at 126 bp (Fig. 1 and Fig. 2). These results detected by Waller and Ogata (2000) and Rajagunalan *et al*. (2014).

PCR analysis detection of *Campylobacter* can be utilized as a simple and rapid tool to discriminate stains recovered from different sources (French, 2008 and El-Adawy *et al.,* 2012). Finally, we concluded that prevention and control of *C. jejuni* in turkey farms would reduce the risk of human exposure to *Campylobacter* and is an important food safety issue. Multiplex PCR was found to be more reliable than the conventional cultural methods in species level for identification and differentiation of *Campylobacter* isolates. Further epidemiological studies at different geographical areas, carried to discover the prevalence, magnitude and importance of *Campylobacter* infection in turkey farms.

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التصنيف الجزيئ لمعزولات الكامبيلو باكتر في قطعان الرومي بمحافظات الدلتا

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تم تجميع عدد ٢٤٠ عينة من مزارع الرومى منها عدد ١٥٠ عينة مسحات شرجية وعدد ٩٠ عينة من طيور الرومى المذبوحة من الاسواق المختلفة بمحافظات الدلتا لعزل ميكروبات الكامبيلوباكتر العينات تم زرعها خلال ٤ ساعات فى الثيوجليكولات ميديا لمدة ٢٤- ٧٢ ساعة ثم زرعها فى اطباق تحتوى على ميديا للكامبيلوباكترتحتوى على ١٠% دم اغنام والمضادات الحيوية الخاصة بالكامبيوباكتروضعت فى جار الزرع اللاهوائى وتم تحضينها فى بيئة تحتوى على ٥% اوكسجين و ١٠% ثانى أكسيد الكربون و ٨٥% نيروجين لمدة ٤٨ ساعات لنمو المستعمرات البكتيرية للكامبيلوباكتر واجراء الاختبارات البيوكيميائية عليها لتصنيف نوع الكامبيلوباكتر الموجود. وبأجراء أختبار البلمرة المتسلسل المتعدد وجد نوعين من الكامبيلوباكتر (الكامبيلوباكتر جيجونای والكامبيلوباكتر كولى) وكانت نسبة عزل ميكروبات الكامبيلوباكتر من الرومى اثناء الدراسة هى ٢٢,٥٪، الكامبيوباكتر جيجونای بنسبة ١٥,٤% ووالكامبيلوباكتر كولى ٧% وكانت اعلى نسبة للعزل من عينات الكبد ٣٠% يليها عينات الجلد ٢٦,٧% ثم المسحات الشرجية ٢١,٣٪، واخيرا من الأمعاء ١٦,٧٪ وتعد نسبة الاصابة الاعلى للكامبيلوباكتر فى الاحشاء الداخلية نتيجة التلوث اثناء عملية التخلص من الاحشاء الداخلية للطائر بعد الذبح اويحدث التلوث الغير مباشر عن طريق الايدى او الادوات المستخدمة اثناء عملية الذبح. وبالتحليل الجينى لميكروبات الكامبيلوباكتر المعزولة كانت نسبة العزل ١٩% للكامبيلوباكتر جيوجونای بنسبة ١٣,٨% وللكامبيلوباكتر كولى عند bp323عند (16SrRNA لجين 126bp)

يمكن استخدام تحليل PCR للكشف عن الكامبيلوباكتر كأداة بسيطة وسريعة وحساسة لتمييز المعزولات من المصادر المختلفة ، لا سيما عند استخدامها كتحليل فى استراتيجية التحكم للكامبيلوباكتر جيوجينای في مزارع الرومي للحد من خطر التعرض البشري للاصابة بعدوى الكامبيلوباكتروهي قضية هامة لسلامة الأغذية. ويعتبر اختبار البلمرة المتسلسل المتعدد اكثر دقة من الاختبارات التقليدية لزرع الميكروب و تحديد معزولات الكامبيلوباكتر ونحتاج مزيد من الدراسات الوبائية فى مناطق جغرافية مختلفة لاكتشاف انتشار وحجم وأهمية الإصابة بعدوى كامبيلوباكتر في مزارع الرومى.