Traceability of enteropathogenic *E. coli* in cattle and camel carcasses

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A B S T R A C T

A total of 120 random samples of cattle and camel carcasses (60 0f each) were collected from the different abattoirs located in Qaliubiya governorate. The samples taken from each carcass were represented meat, spleen, liver and kidneys (15 samples of each), the samples were collected directly after slaughtering and evisceration. The collected samples were subjected to bacteriological examination for detection and identification of *E. coli*. Enteropathogenic *E. coli* organisms were 6.67%, 20%, 26.67%, and 40% of the examined samples of cattle meat, spleen, liver and kidney respectively and were 0.0%, 13.33%, 13.33%, and 33.33% of the examined samples of camel meat, spleen, liver and kidney respectively. The isolated strains were investigated by using Multiplex PCR to detect presence of virulent genes (*stx1, stx2 and eaeA)* in each isolated Strain of *E. coli*. O_{15} Positive strain for stx2 gene, O_{26} & O_{111} Positive strains for stx1, stx2 and eaeA genes. O_{44} & O_{126} Positive strains for stx1 gene. O_{55} Positive strain for stx2 gene and eaeA genes. O_{91}, O_{103} & O_{127} Positive strains for stx1 and stx2 genes O_{124} negative strains for stx1, stx2 and eaeA genes. The public health importance of the isolated *E. coli* and the possible sources of contamination of cattle and camel carcasses with *E. Coli* as well as suggestive hygienic measures to improve the quality of carcasses were discussed.

KEYWORDS: *E. Coli*, cattle, camel, carcasses, PCR.

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1. INTRODUCTION

Meat is considered as an important source of proteins and essential amino acids. Due to this rich composition, it offers a highly favorable environment for the growth of pathogenic bacteria. The microbiological contamination of carcasses occurs mainly during processing and handling, such as skinning, evisceration, storage and distribution at slaughterhouses and retail establishments (Gill, 1998 and Abdalla *et al*., 2009). The microbial quality of meat depends up on the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, the temperature and other conditions of storage during distribution (Nychas *et al.*, 2008).

Escherichia coli is considered the most commensally living microorganism in the alimentary tract of nearly all domestic and wild animals as well as human. Enteropathogenic *E. coli* organisms usually lead to severe diarrhea in infants and it may also be the causal organisms in appendicular abscess, peritonitis and cholecystitis (Frazier and Westhoff, 1988 and Mackie and McCartney, 1989). The presence of *E. coli* is thought to give an indication of faecal contamination (enteric pathogens in particular) than the entire group of *Enterobacteriaceae*. (Kagambèga *et al.,* 2011). The food-borne pathogens are grouped into the following categories based on distinct virulence properties, different interactions with intestinal mucosa, distinct clinical syndromes differences in epidemiology and distinct O:H serogroups: Enteropathogenic E.*coli* (EPEC), Enteroinvasive *E.coli*(EIEC), Enterotoigenic *E.coli*(ETEC), Enterohaemorrhagic *E*.coli (EHEC) and Verocyto-toxigenic *E.coli* (VTEC) (FAO and WHO, 1991).

Shiga toxin (stx) producing *E.coli* (STEC) was among the most common causes of food-borne diseases. The role of non O_{157} STEC strains (e.g., O_{26}:H_{11}/H, O_{91}:H_{21}/H, O_{103}:H_2, O_{111}:H, O_{113}:H_{21}, O_{121}:H_{19}, O_{128}:H_2/H, and O_{145}:H_{28}/H) as causes of HUS, bloody diarrhea, and other gastrointestinal illnesses was being increasingly recognized. (Ursula *et al*., 2012 and Son *et al*., 2014). The different *E. coli* serovars which isolated from different meat products by multiplex PCR with

specific primers for *Stx1, Stx2* and eae genes revealed the presence or absence of these genes in the tested isolates. The obtained results showed that the isolates *E. coli* O_{111} and O_{26} proved to have the three genes *(Stx1*, *Stx2* and eaeA genes), Meanwhile O_{119} isolates were positive for Stx1 and *Stx2* genes. In addition, O86 isolates had only*Stx2* (Shawish, 2015). *E. coli* causes illness ranging from gastrointestinal tract-related complications such as diarrhea, dysentery, urinary tract infection, pneumonia and even meningitis (Johnson *et al.,* 2006).

2. MATERIALS AND METHODS

2.1. Collection of samples

A total of 120 random samples of cattle and camel carcasses (60 0f each) were collected from the different abattoirs located in Qaliubiya governorate. The samples taken from each carcass were represented meat, spleen, liver, and kidneys (15 samples of each). Each sample was kept in a separated sterile plastic bag and preserved in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay and examined as quickly as possible. The collected samples were subjected to bacteriological examination to detect *E. coli.*

2.2. Preparation of samples: (ICMSF. 1982).

Twenty five gms were taken aseptically from the examined meat, spleen, liver and kidney samples and transferred aseptically to a sterile homogenizer bag containing 225 mls of sterile peptone water (1%) and homogenized for 2.5 minute at 3000 r.p.m. to provide a diluation 101, then decimal serial dilutions were prepared.

2.3. Screening of Enteropathogenic Escherichia coli (APHA, 1984 and ICMSF, 1996).

2.3.1. Pre-enrichment:

One ml from the original dilution was inoculated into MacConky broth tube supplemented with inverted Durham's tube. The inoculated and control tube were incubated at 37°C /24-48hrs. Tubes showing gas production were considered positive for coliforms.

2.3.2. Enrichment:

One ml from positive MacConkey broth was transferred into Brilliant Green Bile 2% broth tubes supplemented with inverted Durham's tube and incubated at 44± 0.5°C for 18 hours (Eijkman test).

2.3.3. Selective plating:

A loopful from a positive Brilliant Green Bile (2%) broth tube was streaked into Eosine Methylene Blue agar (EMB) incubated at37°C /24; typical colonies of *E. coli* appear greenish metallic with purple center.

2.3.4. Identification of Escherichia coli:

Microscopical examination: Gram negative coccobacilli to medium size rods.
Biochemical identification: Motility test: + ve result (non-motile) (MacFaddin, 2000). Indol production test: + ve result (red ring) (MacFaddin, 2000). Methyl Red reaction: + ve result (red colour) (Ljutov, 1961). Voges Proskaure test: –ve result (no change in color) (Ljutov, (1963). Gelatin Liquefacrion: –ve result (Collins, 1984). Hydrolysis of urea: –ve result (no change in color) (Edwards, and Ewing, 1972 and Krieg, N.R. and Holt, J.G., 1984). Hydrogen sulphide test: –ve result (no change in colour) (Krieg, and Holt, 984). Utilization of citrate: –ve result (no change in colour) (MacFaddin, 1976). Fermentation of sugars: +ve result with lactose (MacFaddin, 1976). Eijkman test: *E. coli* is one of few organisms that produce gas at this temperature (APHA. (1984).

2.3.5. Serological identification:

The isolates were serologically identified according to (Simmon, 1926), by using rapid diagnostic *E. coli* antisera sets (Denka Seiken Co., Japan) for diagnosis of the enteropathogenic types.

2.4. Polymerase Chain Reaction (PCR):

2.4.1. Materials used for PCR:

Reagents used for agarose gel electrophoresis: Agarose powder, Biotechnology grade (Bioshop^R, Canda inc. lot No: OE16323). It prepared in concentration 2% in 1× TAE buffer. Tris acetate EDTA (TAE) electrophoresis buffer (50×liquid concentration) (Bioshop^R, Canada inc. lot No: 9E11854*).* The solution diluted to 1× by adding 1 ml stock solution to 49 ml double dist. Water to be used in the preparation of the gel or as a running buffer. Ethidium bromide solution (stock solution) biotechnology grade (Bioshop ® Canda Inc, Lot No: 0A14667): The stock solution was diluted by 25µl /200ml double distilled water and stored covered at 4ºC. It was used for staining of PCR products that electrophoreses on agarose gel to be visualized by UV light.
Gel loading buffer (6×stock solution) (Fermentas, lot No: ooo56239). The components were dissolved in sterile double distilled water and stored covered with aluminum foil at room temperature. DNA ladder (molecular marker): 100 bp (Fermentas, lot No: 00052518). 5X Taq master (Fermentas): Containing

polymerase enzyme, Magnesium chloride (Mg Cl_2), Deoxy nucleotide triphosphate (dNTP) and PCR grade water. Primer sequences of *E. coli used* for PCR identification system: Application of PCR for identification of shiga toxins (stx1 & stx2) and intimin (eaeA) genes of *E. coli* was performed essentially by using Primers (Pharmacia Biotech) as follow:

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stx1 (F)	5′ ACACTGGATGATCTCAGTGG ′3		Dhanashree and Mallya (2008)
Stx1 (R)	5′ CTGAATCCCCCTCCATTATG ′3	614	
Stx2 (F)	5′ CCATGACAACGGACAGCAGTT ′3		Dhanashree and Mallya (2008)
Stx2 (R)	5′ CCTGTCAACTGAGCAGCACTTTG ′3	779	
eaeA (F)	5′ GTGGCGAATACTGGCGAGACT ′3		Jeshveen et al. (2013)
eaeA (R)	5′ CCCCATTCTTTTTCACCGTCG ′3	890	

2.4.2. DNA preparation from bacterial culture (Kok et al., 1996)

After overnight culture on nutrient agar plates, one or two colonies were suspended in 20 ml of sterile distilled water, and the suspension was then heated at 100ºC for 20 minutes. From this suspension, a 5 µl aliquot was directly used as a template for PCR amplification.

2.4.3. DNA amplification:

Amplification reaction of *E. coli* (Shah et al., 2009). The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany) using 25 µl of PCR mixture containing 3 µl of boiled cell lysate, 250 µM of each desoxynucleotide triphosphate, 1.5 U of Taq DNA polymerase (Biotools, Madrid, Spain), buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl and 3 mM MgCl2, Biotools), 1 µM of the primers mecA-R, mecA-F; 0.8 µM of icaA-R, icaA-F and 0.8 µM of icaD-R, icaD-F. Amplification conditions were: denaturation for 3 min at 94°C, followed by 30 cycles of 94°C for 1min, 58°C for 1 min and 72°C for 1min, with final extension at 72°C for 5 min. Amplified products were analyzed by 1.5% of agarose gel electrophoresis stained with ethidium bromide and visualized and captured on UV transilluminator.

3. RESULTS

Table (1) indicated that Enteropathogenic *E. coli* organisms were 6.67%, 20%, 26.67%, and 40% of the examined samples of cattle meat, spleen, liver and kidney respectively. The identified serovars of pathogenic *E.coli* were O_{111}: H_2 (6.67%) for cattle meat, O_{91} : H_{21}, O_{111} : H_2, O_{127} : H_6 (6.67% for each) in cattle spleen, O_{26} : H_{11} (13.33%) and O44: H18, O_{103} : H_2 (6.67% for each) in cattle liver, O_{127} : H_6 (13.33%) and O_{15}, O26 : H_{11}, O_{111} : H_2, O_{124} (6.67% for each) in cattle kidney.

Table (2) indicated that Enteropathogenic *E.coli* organisms were 0.0%, 13.33%, 13.33%, and 33.33% of the examined samples of camel meat, spleen, liver and kidney respectively. The identified serovars of pathogenic *E.coli* were O_{26} : H_{11}, O_{126} : H_{21} (6.67% for each) in camel spleen, O_{55}: H_7, O_{111} : H_2 (6.67% for each) in camel liver, O_{111} : H_2 (13.33%) , O_{26} : H_{11}, O_{91} : H_{21}, O_{126} : H_{21} (6.67% for each), in camel kidney.

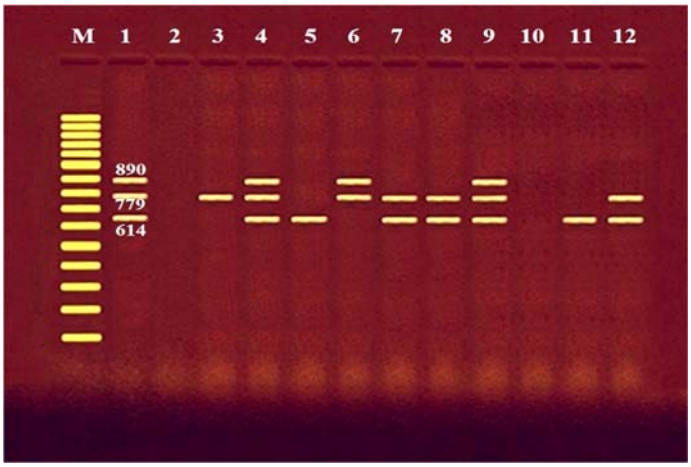
Photo (1) showed agarose gel electrophoresis of multiplex PCR of stx1 (614 bp), stx2 (779 bp) and eaeA (890 bp) genes for characterization of *Enteropathogenic E. coli.* showed that, 10 *E. coli* strains *E. coli* O_{15}, O_{26} , O_{111}, O_{44}, O_{126}, O_{55}, O_{91}, O_{103}, O_{127}& O_{124}, investigated by multiplex PCR to detect presence of virulence genes *stx1, stx2* and *intimin (eaeA*), From the recorded results it was found that *E. coli* O_{15} Positive strain for stx2 gene, *E. coli* O_{26} & O_{111} Positive strains for stx1, stx2 and eaeA genes. *E. coli* O_{44} & O_{126} Positive strains for stx1 gene. *E. coli* O_{55} Positive strain for stx2 gene and eaeA genes. *E. coli* O_{91}, O_{103} & O_{127} Positive strains for stx1 and stx2 genes. While *E. coli* O_{124} considered negative strain for stx1, stx2 and *eaeA* genes. The strains which were positive for eaeA gene which encodes *intimin*, an important binding protein of pathogenic STEC as *E.* coli O_{26}, O_{111}, O_{55} and O_{125} more virulent than other strains not carry this gene and considered more toxigenic and hazardous to consumer health.

Table (1): Incidence and serotyping of Enteropathogenic *E. coli* isolated from the examined samples of cattle meat and offal (n=15).

Tissues / *E.coli* Strains	Meat		Spleen		Liver		Kidney		Strain Characteristics
	No.	%	No.	%	No.	%	No.	%	
O15	-	-	-	-	-	-	1	6.67	EPEC
O26 : H11	-	-	-	-	2	13.33	1	6.67	EHEC
O44: H18	-	-	-	-	1	6.67	-	-	EPEC
O91 : H21	-	-	1	6.67	-	-	-	-	EHEC
O103 : H2	-	-	-	-	1	6.67	-	-	EHEC
O111 : H2	1	6.67	1	6.67	-	-	1	6.67	EHEC
O124	-	-	-	-	-	-	1	6.67	EIEC
O127 : H6	-	-	1	6.67	-	-	2	13.33	ETEC
Total	1	6.67	3	20	4	26.67	6	40	

Table (2): Incidence and serotyping of Enteropathogenic *E. coli* isolated from the examined samples of camel meat and offal (n=15).

Tissues / *E. coli* strains	Meat		Spleen		Liver		Kidney		Strain Characteristics
	No.	%	No.	%	No.	%	No.	%	
O26 : H11	-	-	1	6.67	-	-	1	6.67	EHEC
O55: H7	-	-	-	-	1	6.67	-	-	EPEC
O91 : H21	-	-	-	-	-	-	1	6.67	EHEC
O111 : H2	-	-	-	-	1	6.67	2	13.33	EHEC
O126 : H21	-	-	1	6.67	-	-	1	6.67	ETEC
Total	-	-	2	13.33	2	13.33	5	33.33	



Photograph (1): Agarose gel electrophoresis of multiplex PCR of stx1 (614 bp), stx2 (779 bp) and eaeA (890 bp) genes for characterization of *Enteropathogenic E. coli.* Lane M: 100 bp ladder as molecular size DNA marker. Lane 1: Control positive *E. coli* for stx1, stx2 and eaeA genes. Lane 2: Control negative. Lane 3 (*E. coli* O_{15}): Positive strain for stx2 gene. Lanes 4 & 9 (*E. coli* O_{26} & O_{111}): Positive strains for stx1, stx2 and eaeA genes. Lanes 5 & 11 (*E. coli* O_{44} & O_{126}): Positive strains for stx1 gene. Lane 6 (*E. coli* O_{55}): Positive strain for stx2 gene and eaeA genes. Lanes 7, 8 & 12 (*E. coli* O_{91}, O_{103} & O_{127}): Positive strains for stx1 and stx2 genes. Lane 10 (*E. coli* O_{124}): negative strains for stx1, stx2 and eaeA genes.

4. DISCUSSION

The food-borne pathogens are grouped into the following categories based on distinct virulence properties, different interactions with intestinal mucosa, distinct clinical syndromes differences in epidemiology and distinct O:H serogroups: Enteropathogenic E. *coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterotoigenic *E. coli* (ETEC), Enterohaemorrhagic *E.* coli (EHEC) and Verocyto-toxigenic *E. coli* (VTEC) (FAO and WHO, 1991). The achieved results (table1&2) are nearly similar to those obtained by (Abdalla *et al*., 2009) who examined a total of 384 swab samples (rump, brisket, neck and shoulder) from cattle carcasses and found that *E. coli* (8.86%). Higher results recorded by (Mohamed – amany, 2010) examined 40 liver samples 20 livers each from cattle and camels, *E. coli* could be isolated at rates of 35% for cattle, and 25% for camels. *Escherichia coli* could be seriologically identified as O_{26}, O_{127}, O_{111}, O_{143}, O_{128}. (El-Shamy, 2011) the incidence of *E. coli* isolated out of 25 examined liver samples were 40%, 7(28%) Enteropathogenic, 2(8%). Enterohaemorrhagic and 1 sample (4%) Enterotoxigenic. (Roushdy *et al.*, 1983) who examined 50 liver samples obtained from healthy slaughtered cattle and isolated *E. coli* (42%) and by (Salem–Ghada, S. 2001) who collected offal samples from butcher's shop and street cars and isolated E. coli at (40%) and (60%) in liver samples respectively. (Al-Rhodn 2010) examined 25 liver from slaughtered cows *E.Coli* presence was confirmed biochemically in 53.3% of liver isolates. Enterohemolytic activity was detected in liver. (Wahba 2006) recorded the percentages of *E. coli* on the examined carcass surfaces (fore quarter, hind quarter and brisket) were 55%, 60% and 55%, respectively.

In general, EPEC strains are the major cause for many infantile diarrhea, in typical cases, symptoms appear within 12 to 36 hours. Clinically, EPEC illness is characterized by fever, nausea, vomition and watery stools, which occasionally contain mucous, but without gross blood (Jay, 1997). Furthermore, EPEC was implicated in cases of gastroenteritis, cystitis, colitis, pyelonephritis, and peritonitis as well as food poisoning outbreaks (Sumner *et al.,* 2003). Therefore, EPEC showed to be the first bacterial cause of diarrhea in infants and its proportion may reach 54% (Varnam and Evans, 1991). Though, numerous research efforts have been made during the past decades and in recent years for food borne pathogen detection. Therefore, a detection technique which is reliable, rapid, accurate, simple, sensitive, selective and cost effective has to be developed. In addition, it should be able to detect pathogens in very low concentrations of the samples and must be suitable for in situ real-time monitoring (Arshak *et al.,* 2010).

Application of multiplex PCR for detection of non-O157: H7 STEC virulence genes as (stx1, stx2, eae, hly, etpD, katP6) not only improve the detection efficiency but also increase the accuracy and mentioned that traditional detection approaches for non-O157 STEC are both time and labor consuming in diseases surveillance (*Wang et al., 2013*).

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