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 of 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*. O₁₅ Positive strain for *stx2* gene, O₂₆ & O₁₁₁ Positive strains for *stx1*, *stx2* and *eaeA* genes. O₄₄ & O₁₂₆ Positive strains for *stx1* gene. O₂₅ Positive strain for *stx2* gene and *eaeA* genes. O₀₁, O₁₀₀ & O₁₂₇ Positive strains for *stx1* and *stx2* genes O₁₂₄ 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.

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), Enterohemorrhagic *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 O₁₅₇ STEC strains (e.g., O₂₆:H₁₁/H, O₁₀₁:H₁₁/H, O₁₀₃:H₂, O₁₁₁:H₁₁/H, O₁₁₃:H₂₁, O₁₁₂:H₁₉, O₁₂₈:H₅/H, and O₁₄₅:H₃₈/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 \textit{E. coli} O\textsubscript{111} and O\textsubscript{26} proved to have the three genes (Stx1, Stx2 and eaeA genes). Meanwhile O\textsubscript{119} isolates were positive for Stx1 and Stx2 genes. In addition, O86 isolates had onlyStx2 (Shawish, 2015). \textit{E. coli} causes illness ranging from gastrointestinal tract-related complications such as diarrhea, dysentery, urinary tract infection, pneumonia and even meningitis (Johnson \textit{et al.}, 2006).

2. MATERIALS AND METHODS

2.1. Collection of samples
A total of 120 random samples of cattle and camel carcasses (60 of 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 \textit{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 10\textsuperscript{1}, then decimal serial dilutions were prepared.


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\textdegree 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\textdegree 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\textdegree C /24; typical colonies of \textit{E. coli} appear greenish metallic with purple center.

2.3.4. Identification of Escherichia coli:
Microscopical examination: Gram negative cocccobacilli to medium size rods.

2.3.5. Serological identification:
The isolates were serologically identified according to (Simmon, 1926), by using rapid diagnostic \textit{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\textsuperscript{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\textsuperscript{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 \textsuperscript{R} Canda Inc, Lot No: 0A14667): The stock solution was diluted by 25\mu l /200ml double distilled water and stored covered at 4\textdegree C. 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: 00056239). 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 (MgCl₂), Deoxy nucleotide triphosphate (dNTP) and PCR grade water. Primer sequences of \textit{E. coli} used for PCR identification system: Application of PCR for identification of shiga toxins (stx1 & stx2) and intimin (eaeA) genes of \textit{E. coli} was performed essentially by using Primers (Pharmacia Biotech) as follow:

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Oligonucleotide sequence (5’ → 3’)</th>
<th>Product size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx1 (F)</td>
<td>5′ ACACTGGATGATCTCAGTGG ′3</td>
<td>614</td>
<td>Dhanashree and Mallya (2008)</td>
</tr>
<tr>
<td>Stx1 (R)</td>
<td>5′ CTGAATCCCCCTCCATTATG ′3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stx2 (F)</td>
<td>5′ CCATGACAACGGACGACAGTT ′3</td>
<td>779</td>
<td>Dhanashree and Mallya (2008)</td>
</tr>
<tr>
<td>Stx2 (R)</td>
<td>5′ CCTGTCAACTGAGCAGACCTTTG ′3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eaeA (F)</td>
<td>5′ GTGGCGAATACTGGCGAGACT ′3</td>
<td>890</td>
<td>Jeshveen et al. (2013)</td>
</tr>
<tr>
<td>eaeA (R)</td>
<td>5′ CCCCATTTTTTCCACGTG ′3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 100°C 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 \textit{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 1 min, 58°C for 1 min and 72°C for 1 min, 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 \textit{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 \textit{E. coli} were O₁₁₁: H₂ (6.67%) for cattle meat, O₀₁ : H₂¹, O₁₁₁ : H₂, O₁₂₇ : H₆ (6.67% for each) in cattle spleen, O₂₆ : H₁ (13.33%) and O₄₄: H₁₈, O₁₀₃ : H₂ (6.67% for each) in cattle liver, O₁₂₇ : H₆ (13.33%) and O₁₅, O₂₆ : H₁₁, O₁₁₁ : H₂, O₁₂₄ (6.67% for each) in cattle kidney.

Table (2) indicated that Enteropathogenic \textit{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 \textit{E. coli} were O₂₆ : H₁₁, O₁₂₆ : H₂₁ (6.67% for each) in camel spleen, O₅₅: H₇, O₁₁₁ : H₂ (6.67% for each) in camel liver, O₁₁₁ : H₂ (13.33%), O₂₆ : H₁₁, O₉₁ : H₂₁, O₁₁₁ : H₂, O₁₂₆ : H₂₁ (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 \textit{E. coli}. showed that, 10 \textit{E. coli} strains \textit{E. coli} O₁₅, O₂₆ , O₁₁₁, O₄₄, O₁₂₆, O₅₅, O₀₁, O₁₀₃, O₁₂₇ & O₁₂₄, investigated by multiplex PCR to detect presence of virulence genes stx₁, stx₂ and intimin (eaeA), From the recorded results it was found that \textit{E. coli} O₁₅ Positive strain for stx₂ gene, \textit{E. coli} O₂₆ & O₁₁₁ Positive strains for stx₁, stx₂ and eaeA genes. \textit{E. coli} O₄₄ & O₁₂₆ Positive strains for stx₁ gene. \textit{E. coli} O₅₅ Positive strain for stx₂ gene and eaeA genes. \textit{E. coli} O₀₁, O₁₀₃ & O₁₂₇ Positive strains for stx₁ and stx₂ genes. While \textit{E. coli} O₁₂₄ 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 \textit{E. coli} O₂₆, O₁₁₁, O₅₅ and O₁₂₅ more virulent than other strains not carry this gene and considered more toxigenic and hazardous to consumer health.
Traceability of enteropathogenic *E. coli* in cattle and camel carcasses

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

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Meat Strains</th>
<th>No.</th>
<th>%</th>
<th>Spleen Strains</th>
<th>No.</th>
<th>%</th>
<th>Liver Strains</th>
<th>No.</th>
<th>%</th>
<th>Kidney Strains</th>
<th>No.</th>
<th>%</th>
<th>Strain Characteristics</th>
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<td>E. coli</td>
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<tr>
<td>O15</td>
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<td>1</td>
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<td></td>
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<td></td>
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<tr>
<td>O26 : H11</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>13.33</td>
<td>1</td>
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<td>EHEC</td>
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<tr>
<td>O44: H18</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>O91 : H21</td>
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<td>-</td>
<td>-</td>
<td>1</td>
<td>6.67</td>
<td>-</td>
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<td>-</td>
<td>EHEC</td>
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<td>O103 : H2</td>
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<td>-</td>
<td>1</td>
<td>6.67</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>EHEC</td>
<td></td>
<td></td>
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<tr>
<td>O111 : H2</td>
<td></td>
<td>1</td>
<td>6.67</td>
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<td>6.67</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>6.67</td>
<td>-</td>
<td>EHEC</td>
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<tr>
<td>O124</td>
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<td>O127 : H6</td>
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<tr>
<td>Total</td>
<td></td>
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<td>20</td>
<td>4</td>
<td>26.67</td>
<td>6</td>
<td>40</td>
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Table (2): Incidence and serotyping of Enteropathogenic *E. coli* isolated from the examined samples of camel meat and offal (n=15).

<table>
<thead>
<tr>
<th>Tissues</th>
<th>E. coli Strains</th>
<th>No.</th>
<th>%</th>
<th>Spleen Strains</th>
<th>No.</th>
<th>%</th>
<th>Liver Strains</th>
<th>No.</th>
<th>%</th>
<th>Kidney Strains</th>
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<th>%</th>
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<tbody>
<tr>
<td></td>
<td>O26 : H11</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
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<td>6.67</td>
<td>-</td>
<td>EHEC</td>
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<td></td>
<td>O55: H7</td>
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<td>-</td>
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<td>6.67</td>
<td>-</td>
<td>EPEC</td>
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<td>O91 : H21</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>O111 : H2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>2</td>
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<tr>
<td></td>
<td>O126 : H21</td>
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</table>

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* O15): Positive strain for stx2 gene. Lanes 4 & 9 (*E. coli* O26 & O111): Positive strains for stx1, stx2 and eaeA genes. Lanes 5 & 11 (*E. coli* O44 & O126): Positive strains for stx1 gene. Lane 6 (*E. coli* O35): Positive strain for stx2 gene and eaeA genes. Lanes 7, 8 & 12 (*E. coli* O91, O103 & O127): Positive strains for stx1 and stx2 genes. Lane 10 (*E. coli* O12a): 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 Verocytotoxigenic 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 serologically identified as O26, O127, O111, O145, O26. (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 (Saleem–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-Rhodan 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, vomiting and watery stools, which occasionally contain mucus, but without gross blood (Jay, 1997). Furthermore, EPEC was implicated in cases of gastroenteritis, cystitis, colitis, pylonelphritis, 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).

5. REFERENCES


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