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ISOLATION AND SEROTYPING OF SHIGA TOXIN PRODUCING *E.COLI* FROM RAW MILK IN EGYPT

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ABSTRACT

Shiga-toxin producing *Escherichia coli* (STEC) is an enteric pathogen that have been linked to outbreaks from foodborne and water borne sources. The present investigation was done to study the distribution of O - serogroups of Shiga-toxin producing *Escherichia coli* isolated from milk samples collected from different governorates in Egypt. 158 samples were randomly collected and immediately transferred to laboratory. All samples were cultured and *E. coli* strains were isolated then identification of O serogroups was done by slide agglutination. The isolated *E. coli* strains were positive for incidence of 7 serotypes namely O157, O26, O111, O78, O125, O158, and O127. O157 (12.64%) and O26 (9.48%) were the most frequently identified serogroups. High prevalence of O157, O26 strains and other important serogroups which pose an important public health problem regarding the consumption of raw milk was discussed.

Keywords:

Shiga toxin producing *Escherichia coli*, raw milk, Serotyping.

INTRODUCTION

Escherichia coli (E. coli) is a gram-negative, rod-shaped, flagellated, non-sporulating, and facultative anaerobic bacterium which belongs to Enterobacteriaceae Family. **(Q. Wang *et al.*, 2010).**

Raw milk can be a major source of harmful bacteria to human such as pathogenic *Escherichia coli.* It is possible that milk can be contaminated with a variety of microorganisms from different sources **(Oliver *et al.*, 2005)**.

E. coli is one of these microorganisms, which is a normal inhabitant of large intestine in human and warm-blooded animals. The main source of *E. coli* in raw milk is fecal contamination during milking process along with poor hygienic practices. So, *E. coli* is

generally used as indicator of direct or indirect fecal contamination and the possible presence of enteric pathogens in raw milk **(Kornaki and Johnson 2001).**

E. coli associated with human diseases can be divided into two categories, intestinal and extra-intestinal infections, based on their virulent properties and clinical symptoms. *E. coli* causing intestinal infection is generally called diarrheagenic *E. coli* (DEC), which can be subdivided into six categories, such as enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E.coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC) based on their pathogenic mechanisms and presence of specific virulence genes **(Kaper *et al.,* 2004).**

On the other hand, extra-intestinal pathogenesis caused by *E. coli* (ExPEC) strains can be grouped into three categories, such as uropathogenic *E. coli* (UPEC) causing urinary tract infection (UTI), meningitis-associated *E. coli* (MNEC) and necrotoxigenic *E. coli* (NTEC) which produces cytotoxic necrotizing factor (CNF) **(Kaper *et al.,* 2004).**

Many virulence factors responsible for causing diseases as two phage encoded cytotoxins (*stx1* and *stx2*) **(Kawano *et al.,* 2012),** intimin (*eaeA*) and hemolysin (*hlyA*) **(Slanec *et al*., 2009).**

The most prevalent serotypes with the enteropathogenic *E. coli* (EPEC) are O18, O20, O25, O26, O44, O55, O86, O91, O111, O114, O119, O125 ac, O126, O127, O128, O14 and O158 **(Nataro and Kaper *et al.,*1998)** these serotypes linked epidemiologically to infantile diarrhea. While, the most prevalent serotypes associated with (STEC) human infections are O157, O111, O26, O103, O113, O91, O118, O121, O145, O128 and O146 **(Rahal *et al.,* 2015).**

MATERIAL AND METHOD

Sample collection:

Our study comprised 158 of raw milk samples which were collected randomly from different governorates in Egypt (El - beheira, Tanta, El- monfiya and Asyut) in sterile falcon tubes 15 ml. The samples were transported in ice box directly to laboratory of microbiology Faculty of Veterinary Medicine, Cairo University.

Sample Preparation:

After a well mixing of milk sample, a few ml of the sample were inoculated into Tryptone Soya Broth (TSB) and incubated at 37 ^{o}C for 18 - 24 hrs.

Isolation of *E. coli:*

A loopful from each tube was streaked onto Eosin Methylene Blue (EMB) agar plates and incubated aerobically at 37 ºC for 24 hrs.

The greenish metallic sheen colonies were streaked onto nutrient agar and incubated overnight at 37 ºC for 24 hrs.

Isolation of O157 *E.coli*: a few ml of sample were placed into tryptone soya broth and incubated at 37ºC for 16- 18 hrs. The enriched cultures were sub-cultured into Tellurite (2.5 mg/L) - Cefixime (0.05 mg/L). Sorbitol MacConkey agar (TC-SMAC) and incubated at 37 ºC for 24 hrs. The non-sorbitol fermenting colonies were selected from TC-SMAC plates, and were confirmed by biochemical tests **(You *et al.*, 2006; Lee and Choi, 2006)**.

Serotyping of O 157 *E.coli* and other non O 157 *E. coli* isolates:

Each *E. coli* isolate was serotyped by slide agglutination test by using rapid diagnostic *E. coli* antisera sets (OXOID, UK).

A drop of test latex was dispensed onto a circle on the reaction card, loopfuls of saline were added to the circle and using a loop, a portion of the colony was picked off and emulsified in the saline drop. The test latex and suspension were mixed and rocked in a circular manner observing for agglutination.

Interpretation of result:

Agglutination of the test latex within 1 minute is a positive result. This indicates the presence of *E. coli* serotype O157.

No agglutination occurring within 1 minute is a negative result. This indicates the absence of *E. coli* serotype O157.

Identification of Non O157 serotypes was done in the Serological Laboratory of Animal Health Research Institute (Dokki, Giza.).

RESULT

The recovery rate of *E. coli* from total examined samples was 25.3 (40/158). Samples were positive for incidence of 7 serotypes (O157, O26, O111, O78, O125, O158, O127) the prevalence rates of O157 and O26 serotypes were the highest serotypes which found among serotypes.Two enteropathogenic *E.coli* (EPEC) strains (O78, O158), four enterohaemorrhagic *E.coli* (EHEC) strains (O157, O26, O111, and O125) and one enterotoxigenic *E. coli* (ETEC) strains (O127) were isolated in the study.

Table (1): Prevalence of O157 & non O157 serotypes in different strains isolated from raw milk.

No. of samples	*E. coli* serotype	*E. coli* biotype	No. of isolates/ total no.	%
	O157	**EHEC**	**8**	**5.06**
	O26	**EHEC**	**6**	**3.79**
158	**O111**	**EHEC**	**1**	**0.63**
	O78	**UPEC**	**2**	**1.26**
	O125	**EHEC**	**2**	**1.26**
	O158	**EPEC**	**1**	**0.63**
	O127	**ETEC**	**1**	**0.63**

DISCUSSION

Milk has been associated with health benefits as it contains bioactive peptide, probiotic bacteria, antioxidant, vitamins and highly absorbable calcium **(Bhat and Bhat *et al.*, 2011).** However, the method of production, transportation, handling and sale of milk might be unhygienic which lead to contamination by food poisoning bacteria as *E. coli*.

Raw milk is the main source of STEC transmission in Africa. Serotypes O157, O26 and O111 have also been studied in cattle by several authors **(Pearce *et al.*, 2006; Ekiri *et al.*, 2014).** Our results support previous evidence that cattle represent a major reservoir of *E. coli* serotype 0157 and other non O157 *E. coli*.

E. coli was detected in 25.31% of the examined milk samples which is considered a very high percentage, when compared with previous report (15.9%) in Saudi Arabia **(Al-Zogibi *et al.*, 2015)**. High prevalence was recorded in Ethiopia (29.6%) by **(Garedew *et al.*, 2012).** However other studies reported high prevalence in other products like meat products that range from 9.38% in Egypt **(Mohammed *et al.*,2012)** and 11.6% in Romania **(Bardasi *et al.*,2015)** These variations in the prevalence rate of STEC might be explained by the type of samples examined, their source as well as the method of detection.

In this study *E. coli* O157 was recovered from 8 of 158 samples (5.06%). However in the United Kingdom, the organism was isolated from 2 of 207 cows at slaughter (1%) **(Chapman *et al.*, 1989).** In Spain, O157 serotype was isolated from 1 of 78 calves with diarrhea (1.3%) **(Gonzalez and Blanco *et al.*, 1989)**; and in Germany, it was isolated from none of 47 healthy dairy cows and 2 of 212 healthy bulls (1%) **(Montenegro *et al.*, 1990)**.

O157-STEC was detected in (5.06%) of the milk samples with higher incidence than similar studies in Egypt (2.3%) **(Ahmed and Shimamoto *et al.*, 2014),** and in Saudi Arabia (4.8%) **(Al-Zogibi *et al.*, 2015)**.However, Selim *et al.* **(Selim *et al.*, 2013)** failed to detect any O157-STEC in milk samples in another study in Egypt.

Non O157-STEC isolates were predominant in this study (8.2%). Six different Non O157-STEC O serogroups were detected (O26, O111, O125, O158, O78, O127), which indicates high diversity of *E. coli* serotypes circulation among cattle in the study regions.

The isolation of VTEC 0125, 0111 and 026 from raw milk was similar to the findings of **(Muehlherr *et al.*, 2003)** who detected 12 VTEC strains belonging to the non - O157 VTEC from goat milk. However in our study we obtained 4 VTEC strains.

Most of these serotypes are classified as verotoxigenic serotypes associated with bloody diarrhea or hemolytic uremic syndrome in human **(Johnson *et al.*, 1996; Delannoy *et al.*, 2012)**.

All detected STEC serogroups detected in this study were implicated in human diseases as follow; O157-STEC in cases of Diarrhea in Egypt **(Selim *et al.*, 2013),** O126-STEC, O157-STEC and O158-STEC in urinary tract infection cases in Egypt **(Osman *et al.*, 2012)**.

CONCLUSION

Our study confirmed the risk of different serotypes of pathogenic *E. coli* in raw milk which cause highly potential diseases in human. So we recommend follow hygienic measures during handling raw milk, vaccination against different serotypes of pathogenic *E. coli* and application of HACCP system in dairy farms.

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