Cairo University
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The risk of *Escherichia coli* O157:H7 in Egypt through local and imported beef : special concern to toxin production and virulence gene detection

Thesis presented by

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(Bacteriology, Immunology, and Mycology)

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Abstract:

In this study, 360 samples of beef meat and meat products were collected from different markets, where79 isolates of *E.coli* (21.9%) were isolated. Beef sausage showed the highest rate of isolation (40%) followed by beef oriental sausage; beef luncheon; Beef burgers; minced beef meat; raw beef meat and frozen imported meat (33.3%; 30%; 22%; 16.7% 13.3% and 4% respectively).

In raw beef meat, 8 isolates were serogroupd as O26 (37.5%), O111(37.5%) and untyped *E.coli* (25%). In frozen imported meat 2 isolates were serogroupd as O111. In minced beef meat 10 isolates were serogrouped as O157:H7(20%), O111(10%), O148(20%), O55(20%) and O2(30%).. In beef burgers (11 isolates) were serogrouped as O157:H7(9.1%), O55(18.2%), untyped *E.coli*(27.2%), O1(18.2%) and O111(27.2). In Beef luncheon, 15 isolates were serogrouped into O55(26.7%), O157:H7(13.3%), O158(20%), O101(20%), O26(13.3%) and O1(6.7%). In Beef sausage, 18 isolates were serogrouped as O157:H7(16.7%), O55(16.7%), O1(11.1%), O111(11.1%), O26(27.8%) and O101(16.7%). In Beef oriental sausage, 15 isolates were serogrouped as O157:H7(6.7%), O26(20%), O158(13.3%), O55(13.%), O1(20%) and O111(26.7%).

The occurrence of O157:H7 among different samples revealed that beef sausage showed the highest rate of O157:H7 isolates (6.7%) followed by beef luncheon, minced beef meat, beef oriental sausage and beef burgers (4%, 3.3%, 2.2% and 2% respectively) while Raw beef meat and frozen imported meat showed no occurrence of O157:H7.

The genetic profile of *E.coli* O157:H7 and non O157 isolates using real time PCR was detected. The genetic profile *Stx1+Stx2+eae* was only found in one sample of beef burger. The genetic profile *Stx*2+eae was the highest occurrence in non O157 *E.coli* .

All samples positive to VTEC using sELISA were positive to real time PCR test and also clarified the genetic profile of the positive samples.

Only two samples negative to sELISA were positive to real time PCR and its genetic profile was eae and isolates obtained were serogrouped as untyped *E.coli* isolated from raw meat and O26 from beef

It was concluded from this study that the presence of *E.coli* O157:H7 and non O157 producing *VT* in meats products indicates improper or insufficient hygiene management both at the farm and during the slaughtering and meat handling. Immunological-based methods as sELISA was proved to be rapid screening test for the detection of foodborne bacterial pathogens and their toxins. Real Time PCR was considered as the best detection of VTEC in different food samples and in combination with serotyping help in epidemiological study.

Dedication

Dedicated To:

The soul of my father,

My mother,

My sisters & brother

And

my small family(My husband & my lovely son)

ACNOLOEDGEMENT

I wish first to thank ***Allah*** *for helping me to complete this work and supported me with his blessing and unlimited care.*

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LIST OF ABBREVIATIONS

Bp	Base pair
Cacl2	Calcium chloride
CDC	Center for disease control and prevention
CFU	Colony forming Unit.
CFU/g	Colony forming Unit/gram
DNA	Deoxyribonucleic acid
DNTPs	Dexoy Nucleotide Triphosphate.
E.coli	*Eschericia coli*
eaeA	Intimin
EAEC	Enteroaggregative *E.coli*
EHEC	Enterohaemorrhagic *E.coli*
EIEC	Enteroinvasive *E.coli*
ELISA	Enzyme-linked immune assay
EMB	Eosine Methylene Blue Agar
EMM	Expermintal master mix
EPEC	Enteropathogenic *E.coli*
EU RL	European reference lab.
FAM	6-carboxy-fluorescein
g	Gram
HC	Hemorrhagic colitis
HCl	Hydrochloric acid
HUS	Hemolytic uremic syndrome
hylA	Hemolysin toxin protein
ICMSF	International Commission on Microbiological Specifications for Foods.
IMS	Immuno magnetic separation
KCl	Potassium chloride
LB	Luria-Bertani media
LPs	Lipopolysaccharide
LT	Heat-labile enterotoxin
$MgCl_2$	Magnesium chloride
MNEC	Meningitis neonatal associated *E.coli*
MR	Methyl red
MTSB	Modified tryptic soy broth
Nacl	Sodium chloride
Nm	Nano meter
PCR	Polymerase Chain Reaction.
rpm	Revolution per minute
sELISA	Sandwich Enzyme-linked immune assay
SMAC	Sorbitol MacConkey agar
ST	Heat-stable enterotoxin
STEC	Shiga toxin *E.coli*
Stx1	Shiga toxin 1
Stx2	Shiga toxin 2
TAMRA	6-carboxytetramethyl-rhodamine
Taq	Thermos aquaticus.