ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Detection of *Escherichia coli* in Frozen Meat, Liver, Heart and Kidney Imported in to Egypt.

Abeer E. Abdelaziz[2]*, Osman M. Hamed[1], and Mohamed W. Ghafar[1].

[1]Department of zoonoses, faculty of veterinary medicine, Cairo university, Egypt.
[2]Food microbiology department, central public health laboratories, abdien, Egypt.

ABSTRACT

Escherichia coli is a type of bacteria that lives in human and animal intestine. Most types of *E. coli* are harmless, but some can cause diseases, as food borne illness. This study aimed to determine the prevalence of pathogenic *E. coli* in frozen meat, liver, heart and kidneys imported in to Egypt from certain countries, and in some food poisoning human cases. A total of 1363 imported frozen food samples (962 frozen meat, 281 liver, 69 heart and 51 kidneys) imported from different countries. These samples were collected from different ports of entry as Alexandria Port, Port Said Port and Cairo airport. Sixty samples of human including (47vomitus and 13 stools) were also examined in this study. ISO-16649:2002 used for isolation of *E. coli*. *E. coli* contamination from the 1363 samples of frozen imported meat, liver, heart and kidney was [meat 7.7 % (74/962) and liver 13.9 % (39/281)] while it was 14.5 % (10/69) from the heart and 13.7 % (7/51)] from kidney. All human samples were negative for *E. coli.* The presence of pathogenic strains of *E. coli* in buffalo and beef imported frozen meat and organs showed their ability to cause systemic infections which may appear to be threat to the public and a health concern.

Keywords: pathogenic *Escherichia coli*; ISO; VIDAS; imported; meat; liver; heart; kidney.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Food borne diseases represent an important public health concern all over the world either in undeveloped or developing countries which result in illness, death and economic losses. In undeveloped countries there are more than one billion cases of gastroenteritis and up to 5 million deaths annually [1]. Pathogenic *Escherichia coli* is considered one of the most important food borne pathogens of public health associated with beef and buffalo meat.

E. coli is a part of normal intestinal microflora of many warm blooded animals including human and its presence in food is an indicator reflecting fecal contamination [2]. The nonpathogenic *E. coli* strains can give benefits to the host by producing vitamin K and preventing colonization of the intestine with pathogenic bacteria [3]. Raw and undercooked meat are commonly encountered with the bacterium and have been identified as potential sources of Shiga Toxin-producing *E. coli* (STEC). Contamination of food with such organism occur either during primary production, slaughtering or during further processing and handling [4].

Frozen imported meat and organs such as liver, heart and kidney are considered sources of nutrition which have become accepted products and consumed all over the Arabic World. Egypt is one of the countries that depend mainly on importation of frozen meat and organs to face continuous increasing of human population and shortage of local animal protein.

There are only few reports of incidence of pathogenic *E. coli* in imported frozen buffalo, beef meat and liver sold in retail markets but there is no any study or reports of incidence of pathogenic *E. coli* in imported frozen meat and organs as original packets which assess the contamination of these meat and organs in country of origin so the present study was undertaken with the aim to study the presence and characterization of *E. coli* in frozen meat, liver, heart and kidney imported from different countries into Egypt and to expose their role in causing food poisoning; so that, we can evaluate different sources of importation aiming to protect the Egyptian consumers from foodborne illness. The recovered *E. coli* isolates were subjected to biochemical characterization and serotyping to identify different kind of pathogenic strains of *E. coli.*

MATERIALS AND METHODS

Samples

Food samples

A total of 1363 (962 meat, 281 liver, 69 heart, 51 kidney) frozen imported samples were collected from their original packets while lots are in primary destination (Cairo Airport, Port Said Port, Alexandria Port) before market distribution. All samples were transferred refrigerated and under aseptic conditions to Food Microbiology Laboratory at Central Public Health Laboratories for further processing and testing.

Human samples

Sixty (47 vomitus and 13 stool) human samples were collected from hospitals in a sterile leak-proof containers from cases of food poisoning with history of consumption of eating cooked frozen imported meat and liver. Samples were transferred under aseptic conditions to Clinical Microbiology Laboratory at Central Public Health Laboratories for further processing and testing.

Isolation and identification of *E. coli* (Food samples)

Culture media and supplement used in the study were procured from Oxoid, United Kingdom. The standard protocol described in ISO 16649-2002[5] was adopted for the isolation of *E. coli* spp. from buffalo, beef meat, liver, heart and kidney. Briefly, 10 g of each type of sample was mixed well in sterile plastic bags and transferred to 90 ml maximal recovery diluents. Subsequently 1ml of sample suspension was transferred to two Petri dish then **Tryptone Bile X- Glucuronide Agar (TBX)** media was poured into Petri dish followed by 24h of incubation at 44°C. Typical colonies on (TBX) media (green colonies) were picked and streaked further on nutrient agar for purification. The pure cultures were picked for production of indol, methyl red test,

Vogues Proskauer test, utilization of citrate and urea's test. The colonies identified as *E. coli* were subjected to serotyping to investigate the pathogenic strains.

Detection of *E. coli* O 157 including H7 by VIDAS [6]

Twenty five grams of the sample was added to 225 ml pre warmed buffered peptone water with 2mg vancomycin, then homogenized using stomacher, incubated for 24 h at 41.5°C. Five hundred micro liter of the enrichment broth was transferred into the sample well on the strip. The strip was heated for 5 minutes in the Heat and Go, then removed and left to cool for 10 minutes then VIDAS assay was performed. The test is confirmed by using the enrichment broth within 48 hrs following the end of incubation of the enrichment broth. Immune-concentration with VIDAS ICE was performed. Loopful of immune concentration of ice was streaked onto Sorbitol MacConkey agar. The plates were incubated for 18-24 h at 37°C. *E. coli* O157 is Sorbitol negative. Suspected colonies were identified and picked for biochemical confirmation and Serotyping.

Isolation and identification of *E. coli* (Human samples)

Culture media and supplement used in the study were procured from Oxoid –United Kingdom, The standard protocol described in Bacteriological Analytical Manual [7]. The smear of stool sample or vomitus was taken and streaked directly on MacConkey agar, and then the plates were incubated at 37^{o}C for 24 hours. The typical colony (lactose fermenting colony) was picked for biochemical confirmation.

Serotyping of *E. coli* isolates

Cultures identified as *E. coli* were serotyped by *E. coli* antisera at Clinical Microbiology Lab in Central Public Health Laboratories, Ministry of Health, Egypt.

Statistical analysis: Performed using the statistical software package SPSS for Windows (Version 16.0; SPSS Inc., Chicago, IL, USA). The significance difference of *E. coli* species prevalence, among potential exporting countries, was evaluated by chi-square test. Post hoc test for determination of source of difference was performed according to [8].

RESULTS

In the present study, out of 1363 frozen imported buffalo, beef meat, liver, heart and kidney samples, [meat 7.7 % (74/962), liver 13.9 % (39/281)], 14.5 % (10/69) from the heart and 13.7 % (7/51)] from kidney were positive for *E. coli* but all human samples were negative for *E. coli.* All the isolates revealed characteristic features of *E. coli* producing green colonies on TBX media. On preliminary biochemical characterization, they revealed characteristic IMVC pattern and urea's negative. A higher prevalence of (14.5%) was observed among the samples of imported frozen heart, followed by (13.9%) in liver and (13.7%) in kidney.

ISSN: 0975-8585

Table 1: Occurrence of *E. coli* detected in frozen meat, liver, heart, and kidney samples collected from lots imported into Egypt.

Country of Origin	Sample								Total tested	Total +ve (%)
	Meat		Liver		Heart		Kidney			
	# tested	# +ve (%)	# tested	# +ve (%)	# tested	# +ve (%)	# tested	# +ve (%)		
Brazil	552	10 (1.8)	3	0 (0)	15	4 (26.7)	15	2 (13.3)	585	16 (2.7)
India	293	63 (21.5)	NA	------	2	1 (50)	NA	------	295	64 (21.7)
Australia	62	1 (1.6)	12	0 (0)	NA	------	NA	------	74	1 (1.4)
Canada	21	0 (0)	5	0 (0)	NA	------	NA	------	26	0 (0)
America	18	0 (0)	258	39 (15.1)	51	5 (9.8)	36	5 (13.9)	363	49 (13.5)
South Africa	9	0 (0)	NA	------	NA	------	NA	------	9	0 (0)
New Zealand	4	0 (0)	3	0 (0)	1	0 (0)	NA	------	8	0 (0)
Argentina	2	0 (0)	NA	------	NA	------	NA	------	2	0 (0)
Netherland	1	0 (0)	NA	------	NA	------	NA	------	1	0 (0)
Total	962	74 (7.7)	281	39 (13.9)	69	10 (14.5)	51	7(13.7)	1363	130 (9.5)

ISSN: 0975-8585

Table 2: Recovered *E. coli* strains from imported frozen samples in relation to country of origin.

Country of Origin	Sample												Total tested	Total +ve (%)
	Meat			Liver			Heart			Kidney				
	# tested	# +ve	serovar (#)	# tested	# +ve	serovar (#)	# tested	# +ve	serovar (#)	# tested	# +ve	serovar (#)		
Brazil	552	10	O:26 (2) O:44 (1) O:86 (1) O:103 (1) O:114 (1) O:119 (1) O:125 (2) O:142 (1)	3	0	-----	15	4	O:26 (1) O:86 (2) O:119 (1)	15	2	O:26 (1) O:114 (1)	**585**	**16**
India	293	63	O:18 (4) O:25 (3) O:26 (8) O:44 (1) O:86 (6) O:103 (5) O:111 (8) O:114 (6) O:115 (2) O:118 (3) O:121 (7) O:125 (8) O:145 (1) O:157 (1)	NA	NA	NA	2	1	O:26 (1)	NA	NA	NA	**295**	**64**
Australia	62	1	O:26 (1)	12	0	-----	NA	NA	NA	NA	NA	NA	**74**	**1**
Canada	21	0	-----	5	0	-----	NA	NA	NA	NA	NA	NA	**26**	**0**
America	18	0	-----	258	39	O:26 (6) O:39 (1) O:44 (2) O:86 (5) O:103 (6) O:111 (7) O:114 (6) O:119 (5) O:157 (1)	51	5	O:26 (1) O:44 (1) O:55 (1) O:125 (1) O:159 (1)	36	5	O:26 (3) O:142 (2)	**363**	**49**
South Africa	9	0	-----	NA	NA	NA	NA	NA	NA	NA	NA	NA	**9**	**0**
New Zealand	4	0	-----	3	0	-----	1	0	-----	NA	NA	NA	**8**	**0**
Argentina	2	0	-----	NA	NA	NA	NA	NA	NA	NA	NA	NA	**2**	**0**
Netherlands	1	0	-----	NA	NA	NA	NA	NA	NA	NA	NA	NA	**1**	**0**

ISSN: 0975-8585

Table 3: Detection of *E. coli* in clinical samples collected from people having food poisoning.

Clinical sample	No of samples tested	No of positive samples	Percentage of positive samples
Vomitus	47	0	0
Stool	13	0	0
Total	**60**	**0**	**0**

The general chi-square test showed a significant difference between the occurrence of isolated *E. coli* among the potential meat exporters (Brazil, India, Australia and USA). The post hoc test with the adjusted p-value of 0.00625 (level of significance), due to multiple testing, showed that India and Brazil had significant prevalence of *E. coli* with 21.7% and 2.7%, respectively followed by USA with 13.5% and finally by Australia with 1.4%.

Country *E. coli* Cross tabulation

			E. coli		Total
			Positive	Negative	
Country	Brazil	Count	16	569	585
		% within country	2.7%	97.3%	100.0%
		Adjusted Residual	-7.8	7.8	
	India	Count	64	231	295
		% within country	21.7%	78.3%	100.0%
		Adjusted Residual	7.7	-7.7	
	Australia	Count	1	73	74
		% within country	1.4%	98.6%	100.0%
		Adjusted Residual	-2.5	2.5	
	America	Count	49	314	363
		% within country	13.5%	86.5%	100.0%
		Adjusted Residual	2.7	-2.7	
Total	Count		130	1187	1317
	% within country		9.9%	90.1%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	91.249[a]	3	0.000
Likelihood Ratio	95.551	3	0.000
Linear-by-Linear	20.649	1	0.000
Association			
N of Valid Cases	1317		

a. 0 cells (0.0%) have expected countless than 5.
The minimum expected counts 7.30.

Country	P_value *E. coli*	Exact p-value
Brazil	.000000	0.00625
India	.000000	
Australia	.011406	
America	.006528	

ISSN: 0975-8585

DISCUSSION

During the past 23 year, many of human illness outbreaks have been traced worldwide to consumption of under cooked ground beef and other beef products contaminated with Shiga toxin-producing *E. coli* (STEC). Although several routes exist for human infection with STEC, beef remains a main source [9]. The results given in table (1) indicate that imported frozen buffalo meat samples from India are the most frequently contaminated by *E. coli* (21.5%), followed by imported frozen beef meat from Brazil (1.8%) and the imported Australian frozen beef meat were the least frequently contaminated (1.6%). All imported meat is not accepted by 7.7%, this result is lower than the result obtained by [10] who mentioned that the percentage of *E. coli* in her examined samples was 22.45% from 100 samples of frozen imported meat in Assuit city also our results are lower than the result obtained by [11] who examined 120 samples of frozen imported meat collected from meat section in governmental markets in Cairo governorates and the percentage of *E. coli* was 33.4%. and also lower than results obtained by **Amira, 2014** [12]**(11.7%) and Mayada, 2014**[13] **(38.7%) and Farhan, 2014[14] (30.43%).** These results returned to many factors as high contamination in examined frozen meat may be caused by cross contamination through meat handler also the environmental condition in places where the meat storage or sold because the previous researcher collected their samples from shops and super markets but we collect our samples from ports of entry as original packets without any touching of the meat tissue in addition to differences in number of samples and technique of isolation. Our results are nearly the same of the result obtained by (**Hassan, 2013)** [15] whose result of isolation of *E. coli* was 7.1%. But our result is higher than results obtained by (**Hassan, 1991)[**16] whose result of detection of *E. coli* was 6.6%.The result in table (1) also indicate the percentage of *E. coli* in buffalo meat was 21.5%, this result is slightly higher than result obtained by (**Ahmed, 2015**)[17] whose result was 20%.

In Egypt, the consumption of liver is usually done by simple ,rapid and roasting with or without spices ,this way may be insufficient for killing of pathogenic *E. coli* , so this study assess the presence of it through the data given in table (1) which indicate that the highest presence of *E. coli* was in Imported frozen American liver samples (15.1%), this is may be due to contamination in slaughter house in country of origin also USA analyze the liver for presence of *E. coli* O157 only and neglect other species. Our result for liver is lower than results obtained by (**Doaa, 2011)** [18] whose results were 20%, this attributed to many things like number of samples, different technique for isolation and handling of samples. The data given in tables (1) indicate that the highest contamination by *E. coli* was in imported frozen Brazilian heart samples (26.7%) followed by American heart samples (9.8%) and also the imported frozen American kidney are the highest samples for isolation of *E. coli* (%13.9%) followed by Brazilian kidney samples (13.3%). Our result of kidney is higher than results obtained by (**Doaa, 2011) [**18] who examined 10 samples of frozen kidney and all was negative for *E. coli.*

The data given in table (2) indicates the serovars of different types of pathogenic *E. coli* which isolated from examined samples; the first type is enterotoxigenic *E. coli* which include (O114, O125, O115, and O25).This type produce two types of enterotoxin, one of them is similar to toxin of cholera in structure and function, it is considered the main cause of diarrhea in children in the developing countries and the most common cause of travelers' diarrhea [19].

The second isolated type is enteroinvasive *E. coli* (EIEC) which includes O121, O145, O124, and O142. This type causes a syndrome that is identical to shigellosis with profuse diarrhea and high fever [20]. This result is the same of result obtained by (**Hassan, 2013**) [15] who isolate O145 from his examined samples.

The 3rd type is enterohaemorhagic *E. coli* (EHEC) which includes (O157, O111, O26, O55, O103), causes bloody diarrhea, hemolytic syndrome and sudden kidney failure. It is moderately invasive and possesses a phage encoded Shiga toxin that can elicit an intense inflammatory response [21].

The 4th type is enteroagregative *E.coli* (EAEC) which includes O111, O86, causes acute and chronic diarrhea. These serotypes produce a hemolysin and an enterotoxin similar to that of ETEC [22]. This result is the same result obtained by (**Samaa, 2012)** [23] who isolate O86 from her examined samples.

The fifth type is enteropathogenic *E. coli* (EPEC) which includes (O18, O26, O114, O119, O125, O142, O44, O111), causes diarrhea .This virotype has virulence factors that are similar to those found in *Shigella*, EPEC cells are moderately invasive (i.e. they enter host cells) and lead to an inflammatory response [24]. Most of our isolated *E. coli* strains from frozen imported meat is similar to results obtained by (**Shasha, 2008) [11]** whose isolated strains were O157, O26, O25, O145 from one hundred twenty samples.

However our study revealed that all human samples were negative for *E. coli* as mentioned in table (3) but this doesn't mean that transmission of *E. coli is* impossible but this may be due to sampling differences or number of sample examined.

These results call for urgent attention to imported animal products so as not to harm Egyptian consumers. The statistical analysis results for *E. coli*, highest prevalence in meat exported from India and Brazil followed by USA and finally by Australia with the lowest prevalence, should act as a guide for concerned authorities about the hygienic measures applied in these countries and be a corner stone in the future plane of importing meat and meat products from them.

ACKNOWLEDGMENT

Great thanks are due to Dr. Tamer F. Ismail, Lecture of Animal, Poultry and Environmental Hygiene, Department of Veterinary Hygiene and Management, Cairo University, for his help in the statistical analysis part of this study.

REFERENCES

[1] Gould G W, Russell N J. In: Russell and Gould, editors. Food preservatives (2nd ed), New York: Kluwer Academic/Plenum Publishers. (2003); 1-13.
[2] Singleton P. Bacteria in Biology, Biotechnology and Medicine (5th ed.). Wiley (1999); pp. 444–454.
[3] WHO, Report of WHO Scientific Working Group Meeting, World Health Organization, Geneva, Switzerland. (1998).
[4] Newland J W, Strockbine N A, Miller F F A, O'Brien D and Holmes R K. Microbiol. Rev. (1987)**;** 51:206-220.
[5] ISO-16649. Microbiology of food and animal feeding stuff -Horizontal method for the detection of *Escherichia coli*. (2002)**.**
[6] VIDAS UP *E. coli* O157 including H7 (2010). Method certified AFNOR validation (Bio – 12/25-05/09).
[7] Feng, p.; Weagant,S D and Jinneman,k. *E. coli* In Bacteriological Analytical Manual, 8th ed. Revision A. Gaithersburg,M. D: U.S. Food and Drug Administration, AOAC International. (1998).
[8] Garcla-pErez M A & NUNez-antOn V. Cellwise residual analysis in two-way contingency tables. Educational and psychological measurement, (2003)**;** 63(5), 825-839
[9] Morabito, S.; Karch, H.; Mariani-Kurkdjian, P.; Schmidt, H.; Minelli, F.; Bingen, E.; Caprioli, A. J. Clin Microbiol. (1998); 36:840-842.
[10] Nawal M Said. (1991). Studies on market frozen meat in Assuit city, PhD thesis, Assuit University, Fac., Of Vet., Med., Dep. of Meat Hygiene, Egypt.
[11] Shasha F A (2008). Characterization of certain bacteria and fungi associated with A lime layer of frozen meat, B V Sc. thesis, Fac., Of Vet., Med., Alexandria University. Dep., of Microbiology, Egypt.
[12] Amira A I. (2014). Molecular Characterization of multi drug resistance *salmonella enterica* and Pathogenic *E. coli* isolated from meat in Delta Area.MVSc thesis, Kafr ELshikh University.Fac., Of Vet., Med, Dep. Of Microbiology, Egypt.
[13] Mayada Gwida M, Hotzel, H, Geue L, Tomaso H...International scholary Research Notices, (2014) Nov 11: 565671.

[14] Farhan R S et al., Am. J. anim. of vet Sci. (2014): 9(4): 245-251.
[15] Hassan M M A. (2013): Bacteria associated with fungal contamination in frozen beef meat, MD thesis, Fac., Of Vet., Med., Alexandria University, Microbiology Dep., Egypt.
[16] Hassan K M A. (1991). Some Studies on frozen meat, PhD thesis, Zagazig University, Banha branch, Moshtohor, Fac., Of Vet., Med., Dep. of Food Hygiene and Control, Egypt.
[17] Ahmed A S (2015). Quality of native and imported meat in the Egyptian market, M V Sc thesis, Cairo University, Fac., Of Vet., Med., Dep., Of Food Hygiene and Control, Egypt.
[18] Doaa M A I. (2011). Gram negative bacteria in Variety Meats .M V Sc thesis, Beni-Suef University, Fac., Of Vet., Med., Dep.Of Food Hygiene and Control, Egypt.
[19] WHO, (2010). Enterotoxigenic *Escherichia coli* (ETEC). WHO. int. 2010 -12- 08. Retrieved 2014-06-05.
[20] Lan R, Alles MC, Donohoe K, Martinez MB, Reeves PR. Infect. Immun. (2004). 72 (9): 5080-8. doi:10.1128/IAI.72.9.5080-5088.
[21] Chapman P A ; Siddons C A.; Wright D J et al., Epidemiol. Infect. (1993); 111:439.
[22] Harrington S M, Dudley EG, and Nataro JP. FEMS Microbiol Lett, (2006); 254(1): p. 12-8.
[23] Samaa S Abdelmonem. M V Sc. Thesis, Cairo University, Fac., Of Vet., Med., Dep., of Food Hygiene and Control, Egypt. (2012)
[24] Blanco J, Blanco M, Gonzalez E A. et al., Eur. J. Epidemiol. (1993); 9:489. [PubMed].