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INCIDENCE OF E. COLI IN MEAT AND EDIBLE OFFALS OF RABBIT CARCASSES AND THEIR PUBLIC HEALTH SIGNIFICANCES

(With 4 Tables)

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تواجد الميكروب القولونى فى لحم وبعض أعضاء الأرانب المذبوحة وعلاقتها بالصحة العامة

عزه على حسين التابعى

تم دراسة تواجد الميكروب القولونى فى لحوم الأرانب وكذلك فى بعض الأعضاء الداخلية المستساغة للمستهلك وذلك للفحص الميكروبيولوجى لتواجد الميكروب القولونى فى 20 أرنب مذبوح طازج و20 أرنب مجمد معبأ وكل يحتوى على بعض الأعضاء 0 وقد وجد أن نسبة تواجد الميكروب القولونى فى العينات المأخوذة من الأرانب المذبوحة الطازجة 40%، 35%، 30%، 60% وكان متوسط العد البكتيرى الكلى للميكروب القولونى 8.7×10^2، 5×10^2 ، 3.9×10^2، 4.2×10^3 خلية/جرام بينما نسبة تواجد الميكروب القولونى فى العينات المأخوذة من الأرانب المجمدة كانت 10% ،10 %، 15%، 20%، و متوسط العدد الكلى للميكروب القولونى 3×10^2، 2.8×10^2، 5.2×10^2 ، 2.1×10^3 خلية/جرام فى العضلات والكبد والرئتين والكلى على التوالى بينما لم يتم عزل الميكروب القولونى من القلب من اى من الأرانب المذبوحة والمجمدة 0 هذا وقد تم تصنيف العترات الآتية من العينات الإيجابية وهى: O111, O119, O127, O26, O55, O146. وقد أظهرت أغلبية العترات المعزولة إيجابية لإختبار قدرة التلازن الدموى لكرات الدم المأخوذة من دم الأغنام. وقد تم مناقشة أثر تواجد العترات المعزولة على صحة المستهلك والإجراءات التى يجب إتباعها أثناء الذبح وإزالة الأحشاء الداخلية والتعبئة فى الأرانب وذلك لضمان سلامة المستهلك.

SUMMARY

A total number of 40 freshly slaughter and frozen packed rabbits (20 each), including its edible offals were collected from poultry butcher shops and different markets in Port-Said city. Samples from muscles and edible offals (liver, lungs, heart, and kidneys) were examined microbiologically for presence of *Escherichia coli*. The prevalence of *E. coli* in the examined unpacked fresh rabbit samples was 40, 35, 30 and 60% with mean values of $8.7x10^2$, $5.0x10^2$, $3.9x10^2$ and $4.2x10^3$

CFU/gm, meanwhile the prevalence of *E.coli* in the examined packed frozen rabbit samples was 10, 10, 15 and 20% with mean values of $3.0x10^2$, $2.8x10^2$, $5.2x10^2$ and $2.1x10^3$ CFU/gm in muscles, liver, lungs and kidneys respectively. The heart samples were found to be free from *E. coli* in both fresh and frozen samples. A total of 40 isolates recovered from fresh rabbit samples were identified to Serovars O55(20%), O26(15%), O127(12.5%), O119(30%), and O111(20%), meanwhile *E. coli* belonging to Serovars O146(10%), O55(23.3%), O26(6.7), O127(13.3%), O119(20%) and O111(26.7%) which constituted 30 isolates were recovered from frozen samples. The majority of *E.coli* serotypes recovered from the examined samples showed hemolytic activity. Public health significant of the isolated serogroups and sanitary control of rabbit meat and edible offals for consumer safety were discussed.

Key words: *Rabbit carcasses, edible offals, E. coli.*

INTRODUCTION

Rabbit meat has a highly palatable and digestible quality for consumers all over the world. Rabbit meat is tender and good source of protein, iron and phosphorus, low in total fat, saturated fat, cholesterol and sodium. Inspite of its nutritional and health advantages it has not been incriminated in outbreaks of food borne diseases (Lebas *et al.*, 1997; Dalle Zotte, 2002).

E. coli infection of growing rabbits was the most serious problem among the enteric diseases. In industrial rabbit-fattening farms, enteritis caused by *Escherichia coli* is the main cause of morbidity and mortality in weaned rabbits. The native habitat of *Escherichia coli* is the enteric tract of man and animals, thus its presence in food generally indicates direct or indirect pollution of faecal origin. The pathogenic groups includes enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC). of these, only the first 4 groups have been implicated in food or water borne illness (Levine, 1987; Nataro and Kaper. 1998).

Rabbit edible offals are an important part of rabbit carcasses. Offals are defined as those parts of the carcass which are disassembled on the dressing floor, and which form no part of the carcass when it is finally weighed and transferred to the chillers to undergo cooling. Offals are also often classified by the industry as green and red, depending on their origin. Although there is not complete agreement on the definition

of the terms red and green offals, green offals will be defined as those derived from the digestive tract or those being in contact with the feed or grass, hence the term green. Red offals are those that do not come in contact with the contents of the digestive tract, so they are called red in contrast to the green (grass) offals. Using this classification system the tripe and casings would be called green offals, whereas the liver, kidneys, tongue, spleen, glands and heart would be classified as red offals. Offals, in particular liver have been described, as a route of transmission of organisms to human (Frazier and Westhoff, 1988). Literatures about the microbial quality of rabbit edible offals are rare. *E. coli* has an epidemiological interest and importance as some of which are pathogenic, so it is of great important to through the point on the prevalence and incidence of *E .coli* among rabbit carcasses and edible offals. Therefore, the present work was aimed to determine the incidence of *E. coli* among rabbit carcasses and offals. The public health significant and consumers safety were discussed.

MATERIALS and METHODS

Collection of samples:

A total number of 20 freshly slaughter rabbits, including its edible offals were collected from poultry butcher shops and placed separately into plastic sealed sterile bags. In addition, 20 frozen packed rabbits were collected from different markets in Port-Said city. All samples were transported to the laboratory in an ice box for investigation. Frozen samples were left to defrost in their original container at 4 Cº for 6-12 hrs.

Bacteriological examination:

I-Sample homogenization:

10 grams of each muscles and offals sample (liver, lungs**,** heart, and kidneys) were blended with 90 ml of freshly prepared butter field's phosphate buffer solution**.** Ten fold serial dilutions were prepared. The prepared dilutions were examined bacteriologically for detection of *E. coli.*

II-Isolation and Enumeration of *Escherichia coli*:

The technique recommended by APHA (1992) for isolation and identification of *E. coli* was carried out. One ml of each prepared dilution was inoculated separately onto Lauryl Sulphate Tryptose (LST) broth supplemented with inverted Durham's tubes, and was incubated at

37ºC for 24-48 hrs. Tubes showing gas production were considered positive and recorded.

A loopful from each positive LST broth tube was inoculated onto *E. coli* broth tubes containing inverted Durham's tubes and incubated at 45.5 ± 0.2 ºC for 48 hrs. The tubes were examined at 24 hrs for positive gas production and turbidity and the positive were recorded. Enumeration of *E. coli* was applied by using MPN Table based on proportion of EC. medium tubes in 3 consecutive dilutions according to FAO (1992).

A loopful of suspension from gassing EC. medium tubes was streaked onto Eosin Methylene Blue (EMB) agar plate and MacConkey Sorbitol agar (MACS) as double parallel by using spread plating. The plates were incubated at 37°C for 24 hrs.

III-Biochemical identification:

Biochemical tests were applied to metallic green colored, smooth sided colonies on EMB and to colorless or neutral /gray with smoky center and 1-2 mm diameter Sorbitol negative colonies on MACS according to Quinn *et al.* (2002).

IV-Serological identification:

The isolates were identified serologically by the slide agglutination test using diagnostic polyvalent and monovalent *E. coli* antisera (*Escherichia coli* antisera, Denka Seiken Co., Ltd, Tokyo, Japan), following the manufacturer's specification.

V-Hemolysin production:

E. coli isolates were inoculated onto blood agar plates containing sheep blood (5%) and incubated at 37°C for 24 hrs. The plates were examined for the presence of hemolysis (Beutin *et al.*, 1989).

RESULTS

Table 1: Prevalence of *E coli* in the examined rabbit carcasses and edible offals: (n=20 each)

Examined rabbit Samples	Muscles (n=20)		Edible offals								Total	
			Liver (n=20)		Lungs (n=20)		Heart (n=20)		Kidneys (n=20)			
	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
Unpacked fresh (20)	8	40	7	35	6	30	0	0	12	60	33	33
Packed frozen (20)	2	10	2	10	3	15	0	0	4	20	11	11

NB: The percentage was calculated in relative to the total number of each sample (20).

Table 2: Statistical analytical results of *E coli* count (MPN/gm.) recovered from fresh and frozen rabbit carcasses and edible offals.

		Unpacked fresh samples				Packed frozen samples			
		Muscles	Liver	Lungs	Kidneys	Muscles	Liver	Lungs	Kidneys
Total samples		20	20	20	20	20	20	20	20
ND Samples		12	13	14	8	18	18	17	16
+ve Samples		8	7	6	12	2	2	3	4
Stastics	Min.	2.5×10^2	2.0×10^2	2.0×10^2	4.0×10^2	2.0×10^2	2.5×10^2	2.0×10^2	2.0×10^2
	Max.	4.3×10^3	2.0×10^3	1.1×10^3	4.3×10^4	4.0×10^2	3.0×10^2	1.1×10^3	7.5×10^3
	Mean	8.7×10^2	5.0×10^2	3.9×10^2	4.2×10^3	3.0×10^2	2.8×10^2	5.2×10^2	2.1×10^3
	S.D	1.3×10^3	6.6×10^2	3.5×10^2	1.2×10^4	1.4×10^2	3.5×10^1	5.1×10^2	3.6×10^3
	S.E±	4.9×10^2	2.5×10^2	1.4×10^2	3.5×10^3	1.0×10^2	2.5×10^1	2.9×10^2	1.8×10^3

Min. = Minimum. Max. = Maximum. SE± = Standard Error
SD = Standard Deviation ND = not detected

Table 3: Serovars of *E coli* isolates recovered from the examined fresh unpacked and frozen packed rabbit carcasses

Serovars / Source	Total no. of isolates	O 146		O 55		O 26		O 127		O 119		O111	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Fresh unpacked samples	40	0	0	8	20	6	15	5	12.5	12	30	9	22.5
Frozen packed samples	30	3	10	7	23.3	2	6.7	4	13.3	6	20	8	26.7
Total	70	3	4.3	15	21.4	8	11.4	9	12.9	18	25.7	17	24.3

Table 4: Hemolytic activity of the isolated serovars

E. coli Serovars	No. of isolates	Hemolytic activity	
		No.	%
O146	3	2	66.7
O55	15	12	80
O26	8	4	50
O127	9	9	100
O 119	18	15	83.3
O111	17	17	100
Total	70	59	84.3

DISCUSSION

The present results achieved in Tables 1&2 revealed that the prevalence of *E. coli* in the examined unpacked fresh rabbit samples was 40, 35, 30 and 60% with mean values of $8.7x10^2$, $5.0x10^2$, $3.9x10^2$ and $4.2x10^3$ CFU/gm, meanwhile the prevalence of *E. coli* in the examined packed frozen rabbit samples was 10, 10, 15 and 20% with mean values of $3.0x10^2$, $2.8x10^2$, $5.2x10^2$ and $2.1x10^3$ CFU/gm in muscles, livers, lungs and kidneys respectively. The hearts were free from *E. coli* in both fresh and frozen samples. Ghoniem *et al.* (1971) isolated *E. coli* from slaughtered rabbits at higher rate from the livers (60%), the lower rate was in the lungs (7.15%). However Samaha *et al.* (1993) reported that the incidence of *E .coli* in fresh livers, and hearts was 4.76% and 23.8% respectively. Meanwhile *E. coli* was isolated a lower rate represented 13.6% and 4.5% from livers and hearts respectively from freshly slaughtered apparently healthy rabbits (El-Tabiy, 1998). The present results declared that higher incidence of *E. coli* was recorded in the examined unpacked fresh rabbit samples. Moreover, the achieved results proved that kidneys had a higher incidence of *E. coli and* it was higher in fresh samples. Nearly similar findings were recorded previously by Khalaf-Alla (1993) and Abu-Taleb (1995).

Freezing significantly influenced the rate and extent of microorganisms growth. (Katarzyna and Hanna, 2003). Freezing is an easiest, ancient and most convenient method of food preservation. Freezing stop the bacterial action by halts the activities for spoilage microorganisms in and on food (Archer, 2004). Although food, is not sterilized by freezing but the bacterial count and growth is reduced during the cold storage (Moore and Madden, 2001).

E. coli counts considered as reflection of environmental contamination during slaughter processing and /or product handling, and its count is an index of sanitary quality of the examined rabbit samples. High number can easily give rise to public health hazards (Mercuri and Cox 1976; Kanpelancher 1981).

E. coli is a member of coliform group which are referred as general indicator organisms to measure the potential presence of enteric pathogens in food, also measure the fecal contamination of the food products. Bobbitt (2003) mentioned that the total coliform count of fresh rabbit carcasses was $6.2x10^4$ CFU/gm. In this respect Rodriguez-Calleja *et al.* (2004) reported that the mean coliform count was 1.55 ± 0.72 log CFU/g for rabbit carcasses dressed at a small abattoir and was 2.02±

0.79 log CFU/g for rabbit carcasses stored in Spain supermarkets, he found that the number of positive samples of *E.coli* was 4 from 12 samples in rabbit carcasses slaughtered at small abattoir. Also Maghraby (2005) revealed that the mean values of coliform were $1.7x10^3$ and $2.5x10^2$ in fresh and frozen rabbit carcasses respectively.

The results of biochemical and serological identification of *E.coli* isolates were recorded in Table 3. *E. coli* isolates recovered from fresh samples were belonging to Serovars O55(20%), O26(15%), O127(12.5%), O119(30%) and O111(20%), meanwhile O146(10%), O55(23.3%), O26(6.7), O127(13.3%), O119(20%) and O111(26.7%) serovars could be identified from frozen samples (Table 3). Nearly similar serotype isolates were recorded by Gross (1991) and Brook *et al.* (1994). Ali (1995) recovered 34 isolates of *E. coli* 57.6% from the examined suckling rabbits and were serotyped as 4 isolates (O55), 6 isolates (O119), 14 isolates (O128) and 10 isolates (O126). Most of the isolated *E. coli* serotypes are usually associated with many cases of food borne outbreaks. In this concern, Mossel *et al.* (1995) listed *E. coli* as one of the causes of febrile types of gastroenteritis transmitted by foods. They reported that the O111 and O127 as members of the group containing major virulence factors and they considered some of the O111 is verocytotoxine producers and some as enterohaemorrhagic types, the O127 they tabulated as devoid of toxin production but give rise to attachment effacement lesions in the intestinal mucosa and so profuse diarrhea by adhering to the intestinal epithelium and distorting the microvilli and by causing attaching and effacing lesions. *E. coli* Serovars O111 and O119 were enteropathogenic *E. coli* class I which are responsible for many cases of infantile diarrhea (Jay, 1992). Hussein and Omaye (2003) found that the serogroups belonging to O26, O113, O111, O119 and O166 have caused approximately 30% of the hemolytic uremic syndrome (HUS) in US**.**

The majority of *E. coli* serotypes isolated from the examined samples showed hemolytic activity (Table 4). In this respect, Adesiyun *et al.* (1997) reported that from 94 *E. coli* isolates tested for haemolysis 13.8% were hemolytic**.** Meanwhile, Soliman and El-Tabiy (2006) reported that from 60 *E. coli* isolates tested for hemolytic activity 81.7% were positive. Haemolysin productions have a potential role in virulence of hemolytic *E. coli*. Therefore, the contamination of products with *E. coli* serotypes may results in problems for consumers. Moreover, the genes involved in enterohaemolysin production were carried on the EHEC plasmid (Scotland *et al.*, 1990). Several protein toxins associated

with *E.coli* pathogenesis have been identified, including haemolysins. The production of haemolysins by *E. coli* is associated with extra-intestinal infections in man, and thus haemolysins are considered to play an important role in pathogenesis. (Lai *et al.*, 2000).

Bacteria belonging to the family *Enterobacteriaceae* in rabbit meats are undesirable; their presence may be attributed to contamination of surface of carcasses from exterior of the animals and intestinal contents during evisceration and dressing. Rabbit meat and edible offals need control to safe guard of consumer against food poisoning, outbreaks and to save rabbit carcasses from being spoiled on the market. The slaughterhouse and its regulations, represents a key control point of the livestock production chain. In this context, the noteworthiness of hygienic and sanitary control of rabbit meat slaughtering should be emphasized since the growing interest of consumers in this meat. The rabbit carcasses must be packaged with un separate head while the edible offals (liver, kidneys, lung and heart) should be clean, healthy, and free from gall bladder and packaged separately from the carcasses (E.O.S.Q.C.1996). The use of aseptic packaging of the finished product should be encouraged to avoid post processing environmental contamination as well as keeping the meat frozen during transportation and sale.

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