Animal Health Research Institute,
Port Said Laboratory for Food Hygiene.

A STUDY ON THE OCCURRENCE OF *ESCHERICHIA COLI* IN SOME BEEF PRODUCTS WITH SPECIAL REFERENCE TO E*SCHERICHIA COLI* O157:H7.

(With 4 Tables)

By

ZIENAB I. SOLIMAN and AZZA A. EL-TABIY

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دراسة عن وجود ميكروب الإشيرشيا كولاى فى بعض منتجات اللحوم مع الإشارة إلى عترة O157 :H7

زينب ابراهيم سليمان ، عزه على حسين التابعى

تعتبر منتجات اللحوم المصنعة من أهم المنتجات التي يقبل عليها المستهلك. وقد تتعرض لحوم الذبائح للتلوث بالميكروب القولونى أثناء الذبح وتختلط هذه البكتريا جيدا باللحم عند فرم وتجهيز اللحم لإعداد الأنواع المختلفة من منتجات اللحوم كالبرجر والسجق إلى غير ذلك من منتجات اللحوم المصنعة. ومع أن معظم عترات ميكروب الإشيرشيا كولاى غير ضارة إلا أن هناك بعض العترات قد تشكل خطرا على صحة الإنسان. فعلى سبيل المثال العترة O157:H 7 تذكر دائما مرتبطة بحالات شديدة من التسمم الغذائي وخصوصا في منتجات اللحوم الغير مطهية جيدا وقد تؤدى العدوى إلى حدوث فشل كلوي وخاصة في الأطفال. لذلك تهدف الدراسة الحالية إلى عزل وتقييم انتشار الميكروب القولوني العترة O157 :H7 إلى جانب العترات الأخرى في بعض منتجات اللحوم المصنعة التي تباع في السوبر ماركت. تم تجميع خمسة وعشرون عينة من كل من البرجر ، الفرانكفورتر، الكفتة، اللحم المفروم، والسجق. وقد أسفرت التحاليل عن وجود ميكروب الإشيرشيا كولاى بنسب ٥٦ % ، ٤٠% ، ٩٢% ، ٦٨% ، ٧٢% في عينات البرجر ، الفرانكفورتر، الكفتة، اللحم المفروم، والسجق على التوالي. كذلك تم عزل وتصنيف العترة O157 H:7 من ٦ (٤,٨ %) من اجمالى عدد العينات التى تم فحصها. كما تم تصنيف عترات أخرى من العينات الإيجابية وهى O68 O119, O113, O111, O55, O126. هذا وقد تم مناقشة خطورة وجود العترة O157 :H 7 إلى جانب العترات الأخرى على الصحة العامة وكذلك أهم التوصيات بالنسبة لمستهلكي منتجات اللحوم المفرومة المصنعة والتى تتركز فى الطهى الجيد وضمان وصول الحرارة إلى كافة الأجزاء الداخلية وليس السطح الخارجي وذلك للقضاء على الميكروب.

SUMMARY

A total of one hundred and twenty five samples, twenty five of each beef burger, frankfurter, kofta, minced meat and sausage were collected from Port -Said markets. Samples were examined to isolate and evaluate the prevalence rate of *E. coli* O157:H7 and other *E. coli* serotypes. *E. coli* was detected in burger, frankfurter, kofta, minced meat and sausage samples at a rate of 56, 40, 92 68 and 72%, respectively. Six (4.8%) out of all 125 tested meat products samples were found to be contaminated with *E. coli* O157:H7, ten isolates of *E. coli* O157:H7 could be recovered. A total of 50 *E. coli* isolates recovered from positive samples were identified to serogroups, O55 (30%), O111 (22%), O113 (22%), O119 (16%), O68 (6%) and O126 (4%). The majority of *E.coli* serotypes recovered from the examined samples showed hemolytic activity. The public health significant of the isolated serogroups and consumer's safety were discussed.

Key words: *Escherichia coli, beef products, E.coli 0157:H7.*

INTRODUCTION

Many people enjoy beef burgers, sausages and other meat products, especially during the summer months . However, raw and improperly handled or cooked sausages and beef burgers can harbour harmful bacteria including *Escherichia coli*. The bacteria constituting the species *E. coli* are bacteria that normally live in the intestines of humans and animals. Although most strains are harmless, several are known to produce toxins that can cause diarrhea. 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 and Nataro and Kaper. 1998).

In recent years, it has become apparent that one can contract a rather serious bacterial gastro-enteritis by consuming undercooked ground beef. Scientists have identified a rare but dangerous type of *Escherichia coli*, *E. coli* O157:H7 that is responsible for this foodborne illness. Scientists believed that *E. coli* O157:H7 is a mutant strain that was created when a virus infected benign *E.coli* and gave it a string of DNA from *Shigella*, a bacterium that causes severe bloody diarrhea. In both *Shigella* and *E. coli* O157:H7, as few as 10 germs can cause illness;

by comparison, it takes about a billion *Salmonella* bacteria to make sick (Wong *et al.*, 2000). *E. coli* O157:H7 was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea; the outbreak was traced to under cooked burgers served from a fast food chains (Riley *et al.*, 1983). Since 1983, an increasing in number of *E. coli* O157:H7 have been reported in association with consumption of improperly cooked ground beef (Cohen and Giannella, 1991 *and* Siegler *et al., 1993*).

The organism colonizes in the large intestine and produces one or more of the potent cytotoxins referred to as Shiga-like toxins (SLTs) (O'Brien and Kaper 1998). Although more than 60 *E. coli* serotypes produce SLTs, serotype O157:H7 is the predominant pathogen in the EHEC group and the one associated most frequently with human infections worldwide (Karmali, 1989). These toxins are responsible for severe hemorrhagic colitis in humans. In some persons, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome (HUS), in which the red blood cells are destroyed and the kidneys fail, about 2%-7% of infections lead to this complication (Doyle, 1991).

In a view of the importance of *E. coli* O157:H7 from a food safety stand point, this study was planned to investigate the presence of this agent and other pathogenic *E. coli* serotypes among some selected meat products. The public health significant and consumer's safety were discussed.

MATERIALS and METHODS

One hundred and twenty five samples, twenty-five of each beef burger, frankfurter, minced meat, kofta and sausage were collected from Port- Said markets.

The frozen samples were thawed in their original containers in a refrigerator at 2-5°C. Twenty-five grams of each sample were homogenized with 225 ml of tryptone phosphate broth as a pre-enrichment fluid then incubated for 4-6 hours at 37°C. (Mehlman and Lovett, 1984). Two Mossel's enteric enrichment broth tubes (10 ml) were inoculated each by 1 ml from the pre-enrichment medium. One tube was incubated at 44°C for 24 hours to permit the growth of pathogenic *E.coli*, other than serovar O157. The other tube was incubated at 37°C for 24 hours to permit the growth of *E. coli* O157:H7 as well as other serovars unable to grow at high (44°C) temperature (Mehlman and Romero, 1982). Dilutions of culture in tryptone phosphate broth with

peptone water (0.1%) to 10^{-6} were prepared. About 0.1 ml obtained from appropriate dilution were inoculated in MacConkey Sorbitol agar (MACS) and Eosin methylene blue agar (EMB) as double parallel by using spread –plating . The plates were incubated at 37°C for 24 hours. Randomly selected white and colorless sorbitol negative colonies were picked from MACS and streaked separately onto MACS supplemented with cefixime- tellurite (CT, Difco) (CT- MACS) and onto EMB to purify the colonies. The plates were incubated at 37°C for 24 hours (FDA, 2002). Morphological and biochemical tests were applied to colorless or neutral /gray with smoky center and 1-2 mm diameter sorbitol negative colonies on CT- MACS and to metallic green colored, smooth sided colonies on EMB according to Quinn *et al.* (2002). The isolates were identified serologically by the slide agglutination test using diagnostic polyvalent and monovalent *E.coli* O antisera and H 7 antisera (*Escherichia coli* antisera, Denka Seiken Co., Ltd, Tokyo, Japan) ,following the manufacturer's specification.

Hemolysin production (Beutin *et al.*, 1989)

E. coli isolates were inoculated onto blood agar plates containing sheep blood (5%) and incubated at 37°C for 24 hours. The plates were examined for the presence of haemolysis.

RESULTS

Table 1: Prevalence rate of *Escherichia coli* in the examined meat products samples (n=25 of each).

Meat products	Positive samples	% of Positive samples
Beef burger	14	56
Frankfurter	10	40
Kofta	23	92
Minced meat	17	68
Sausage	18	72

Table 2: Prevalence of *E. coli O157*: H7 among the examined meat products (n=25 of each).

Meat products	Positive samples for serovar O157:H7	
	No.	%
Beef burger	2	8
Frankfurter	0	0
Kofta	1	4
Minced meat	3	12
Sausage	0	0
Total	6	4.8

Table 3: Serovars of *E. coli* isolates recovered from the examined meat products samples.

Serovar / source	O55		O111		O113		O119		O68		O126	
	No	%	No	%	No	%	No	%	No	%	No	%
Beef burger	4	8	3	6	4	8	3	6	1	2	2	4
Frankfurter	1	2	2	4	1	2	0	0	1	2	0	0
Kofta	5	10	4	8	3	6	3	6	0	0	0	0
Minced meat	3	6	2	4	1	2	1	2	1	2	0	0
Sausage	2	4	0	0	2	4	1	2	0	0	0	0
Total	15	30	11	22	11	22	8	16	3	6	2	4

NB: Percentage was calculated according to the total number of the isolates (50)

Table 4: Hemolytic activity of *E. coli* isolates recovered from the examined meat products samples.

E. coli serovars	No. of isolates	Hemolytic activity	
		No.	%
O157 : H7	10	10	100
O55	15	15	100
O111	11	9	81.8
O113	11	7	63.6
O119	8	8	100
O68	3	0	0
O126	2	0	0
Total	60	49	81.7

DISCUSSION

Most enteropathogenic *E. coli* outbreaks have been blamed on ground beef and other meat products such as beef burger, and hot dog (Desmarchelier and Grau, 1997). The present investigation was carried out to evaluate the prevalence of *E. coli* O157: H7 and other *E. coli* serotypes among selected types of meat products.

The overall incidence of *E. coli* in different samples was recorded in Table (1), *E. coli* were recovered from burger, Frankfurter, kofta, minced meat and sausage samples at a rate of 56, 40, 92, 68 and 72%, respectively. In this concern, prevalence of *E. coli* from meat and meat products ranging from 30% to76% have been reported by Doyle and Schoeni, (1987), Gallas *et al.*, (2002) and Gad El-Said *et al.*, (2005). This contamination rate of the present samples indicates unhygienic

practices prevailed in slaughter. Cattle are a major reservoir of these groups of bacteria and ground beef have been the major vehicle of *E. coli* transmission. During slaughter process, meat may become contaminated by fecal contamination during evisceration and through skin or hide during dressing (Desmarchelier, 1997 and Rice *et al.*, 1997). When the meat is ground, fecal organisms on the outside of the meat are mixed throughout the ground beef. Also contamination of meat probably occurs during processing. In this respect, Read *et al.* (1990) reported that ground beef meat- processing plants were heavily contaminated with verocytotoxin *E.coli*. In addition, *E.coli* is an indicator of food safety for dehydrated, frozen and refrigerated food, as *E. coli* does not survive well under such condition (Mossel *et al.*,1979). Therefore, its presence might indicate poor temperature control.

Escherichia coli O157: H7, predominantly originated from beef, is a significant pathogen to the public health and thus, need to be vigorously surveyed in meat products. Lake of sorbitol fermentation within 24 hours has been considered a stable phenotypic character of *E. coli* O157: H7 therefore; MACS was used for differentiation of *E. coli* O157: H7 from other enteric bacteria (March and Ratnam, 1986).

Results of biochemical and serological identification of sorbitol negative *E. coli* isolates revealed that six (4.8%) out of all 125 meat products examined were found to be contaminated with *E. coli* O157:H7 (Table 2), three (8%) out of 25 minced meat samples, two (8%) out of 25 burger samples and one (4%) out of 25 kofta samples. The exact contamination rate may be higher than stated here due to the low isolation rate of the culture methods compared to other immunological and genetical methods. Considerably higher isolation rates of *E. coli* O157:H7 than in this study have been reported elsewhere. In South Africa, it was isolated from a total of 74.5% and in Malaysia from 36% of beef samples (Vorster *et al.*, 1994 and Radu *et al.*, 1998). On the other hand, in some studies beef and beef samples have found to be free (Junghannss *et al.*, 1996, Simmons, 1997 and Uhitil *et al.*, 2001). In another studies it was isolated at low contaminated rate, Pai *et al.*, (1984) reported the presence of *E. coli* O157:H7 in 5 out of 17 beef samples. In USA, *E. coli* O157:H7 was isolated from six (3.7%) out of 164 beef samples (Doyle and Schoeni, 1987), in India, Dutta and Deb, (2000) isolated *E. coli* O157:H7 from two (9%) out of 22 minced beef samples. Also in turkey, Baran and Gulmez, (2005) isolated *E. coli* O157:H7 from three (6%) of ground beef samples. Positive isolation of *E. coli* O157:H7 from beef samples in Egypt was reported by Tanios *et*

al., (2002) from two (6.7%) of minced meat samples and by Gad El-Said *et al.*, (2005) with a rate of 3.95% from meat samples.

Enterohemorrahagic *Escherichia coli* O157: H7 is an important foodborne pathogen, its presence even at low rate (4.8%) in the present study may constitutes dangerous beef products. The ability of *E. coli* O157:H7 to withstand the acidic conditions encountered in various foods have generally suggested that passage through the stomach would be insufficient to inactivate the pathogen. (Naim *et al.*, 2003). In addition, the organism always enters the digestive system within a food matrix, Waterman and Small (1998) postulated that high protein content in food (such as ground beef and boiled egg white) might protect enteric bacteria against the killing effect of gastric acids. The data from epidemiological investigations indicated that as few as 10 to 100 cells of *E. coli* O157:H7 per g of raw ground beef were sufficient to cause illness (Abdul-Raouf *et al.*, 1993). Moreover, Wong *et al.* (2000) believed that treatment with antibiotics is contraindicated for *E. coli* O157 poisoning, since it is when the bacteria die, they release the toxins which produce hemolytic uremic syndrome (HUS), for which there is no cure.

Symptoms of *E. coli* O157 infection include bloody and nonbloody diarrhea, vomiting, and abdominal cramps. Illness resolves typically within 7-10 days .A subset of patients, particularly the young and the elderly, will develop HUS, characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal failure (Russell *et al.*, 2000). In the United States, hemolytic uremic syndrome is the principal cause of acute kidney failure in children, and *E. coli* O157: H7 causes most cases of hemolytic uremic syndrome (Besser *et al.*, 1999). An estimated 73,480 people a year are infected with *E. coli* O157:H7 and about 600 of those cases are fatal, according to the federal Centers for Disease Control and Prevention (Wong *et al.*, 2000). These illnesses and deaths were factors that began changing policy towards foodborne disease. The Food Safety and Inspection Service, declared that raw ground beef contaminated with *E. coli* O157 is adulterated and must be further processed to kill the pathogen or be destroyed (FDA. , 2000) .

While *E. coli* O157: H7 is the most renowned Shiga toxin-producing *E. coli* (STEC) , over 200 different types of STEC have been documented in meat and animals, at least 60 of which have been linked with human disease. A number of studies have suggested that non-O157 STEC are associated with clinical disease, and non-O157 STEC are present in the food supply (Acheson, 2000).

Regarding to other serogroups, as shown in Table (3), O55, O111, O113 and O119 were the most prevalent serotypes recovered from the examined samples with an incidence of 30, 22, 22, and 16% respectively, followed by O68 (6%) ,O126 (4%). Most of the isolated serotypes are usually associated with many cases of foodborne outbreaks and multiple sporadic cases in different part of the world. In this concern, Anathan and Subramaniam, (1995) isolated *E. coli* belonging to serotypes O111 from cases of persistent diarrhea in young children. Enteropathogenic *E. coli* belonging to serotypes O111, O103 and O55 were isolated from patients suffering from bloody diarrhea, which may be accompanied by HUS (Desmarchelier, 1997). Non-O157 STEC, such as O111 has caused large outbreaks and HUS in the United States and other countries. (Acheson, 2000). Moreover, 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.

Blood haemolysis is one of character of virulent *E. coli* (Stephen *et al.*, 1985).

Ten isolates identified serologically as *E. coli* O157: H7 were tested for hemolysis production using sheep blood. All tested isolates were haemolytic. Moreover, the majority of *E. coli* isolates other than O157: H7 isolated from the examined samples showed haemolytic activity (Table 4). In this respect, Adesiyun *et al.*, (1997) reported that from 94 *E. coli* isolates tested for haemolysis 13.8 % were haemolytic. Meanwhile, Gad El-Said *et al.*, (2005) stated that 81.58 % of *E. coli* isolates recovered from meat samples showed haemolytic activity.

The productions of haemolysin have a potential role in virulence of hemolytic *E. coli*. Therefore, contamination of meat products with *E. coli* O157:H7 and other *E. coli* serotypes may results in problems for consumers. There is a close association between enterohaemolysin production and SLT production (Beutin *et al.*, 1998). Moreover, the genes involved in enterohaemolysin production were carried on the EHEC plasmid (Scotland *et al.*, 1990).

The risk of contamination of raw meat products with *E. coli* O157:H7 and other pathogens constitute a major problem for human. The low infective dose *E. coli* O157:H7 present a major threat. Hemolytic uremic syndrome, a disease caused mostly by *E. coli* O157:H7 may cause sever kidney diseases and/or failure among children. The main means of combating this organism are good food hygiene covering activities on farm, in abattoir and minced beef

industries. However, until *E. coli* can be eliminated from meat processing systems, consumers should protect themselves by using safe food practices and advice for those who eat ground beef. Frozen ground beef should be thawed in the refrigerator rather than at room temperature. While thawing and preparing ground beef, raw meat must be separated from ready-to-eat foods. It is not enough to merely brown the outside of a burger; and other meat products .Ground beef should be cooked thoroughly to an internal temperature of at least 160° F (71° C), food safety experts recommends that consumers use a meat thermometer to cook ground beef to ensure that internal temperatures are high enough to kill bacteria. To reduce the risk for cross-contamination, consumers should use soap and hot water to wash hands, utensils, and other surfaces that might have been exposed to raw or undercooked ground beef and other meat products. In addition, consumers should be aware from under cooked burgers and other meat products served from fast food restaurants.

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