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PREVALENCE OF *ESCHERICHIA COLI* WITH SPECIAL REFERENCE TO *E.COLI* O157 IN SOME RETAIL MEAT PRODUCTS AND CATTLE IN ASSIUT GOVERNORATE

(With 3 Tables)

By

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مدى تواجد الميكروب القولونى وخاصة العترة *E. coli* O157 فى بعض منتجات اللحوم المعدة للبيع والابقار بمحافظة أسيوط

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أجريت هذه الدراسة على مائة عينة لبعض منتجات اللحوم المعدة للبيع (٥٠ عينة لنشون و ٥٠ عينة لحوم مفرومة من محلات السوبر ماركت والمطاعم المختلفة) ومائة عينة من براز الأبقار المعدة للذبح من مختلف المجازر بمحافظة أسيوط بهدف معرفة مدى تواجد ميكروب الاشيرشيا كولاى وبالأخص العترة O157 فى هذه العينات. ونظرا للخطورة الشديدة للعترة O157 على صحة الإنسان وتواجدها بأعداد قليلة فى الطبيعة والأطعمة المختلفة وحتى فى براز الأبقار فقد تم استخدام بعض الأوساط الغذائية الحساسة والخاصة لعزل وتصنيف هذه العترة. وقد أسفرت النتائج عن الآتى : تواجد ميكروب الاشيرشيا كولاى بنسب ٣٠% ، ٤٦% ، ٥٥% فى عينات اللنشون واللحوم المفرومة وبراز الأبقار على التوالى كما تم عزل وتصنيف العترة O157 من ٤% و ٥% من عينات اللحوم المفرومة وبراز الأبقار ولم يتم عزله من عينات اللنشون . كما أسفرت نتائج التصنيف السيروأوجى إلى تواجد عترات أخرى غير العترة O157 وهى0119, 055, 0117, 0158, 0114, 0107,

فى العينات التى تم فحصها. هذا وقد تمت مناقشة النتائج والاهمية الصحيـــة والاحتياطـــات الواجب اتخاذها لتقليل أو منع تواجد هذا الميكروب فى منتجات اللحوم .

SUMMARY

One hundred meat products (50 samples of each luncheon and minced beef) and one hundred fecal samples of beef cattle were collected randomly from supermarkets, restaurants and slaughter houses at Assiut Governorate for the presence of *E. coli* especially *E.coli* O157. *E.coli* was detected with percentages 30%, 46% and 55% in luncheon, minced beef and fecal samples respectively. *E.coli* O157 was identified in 4% and 5% of minced beef and fecal samples respectively, but not detected in luncheon samples. *E.coli* O119, O55, O117, O158, O114 and O107 were also identified in the examined samples. The results, the public health significance as well as recommended hygienic measures were discussed.

Key words: *E.coli O157, Meat products, Cattle, Assiut, Egypt*

INTRODUCTION

Escherichia coli is considered as a commensal in the alimentary tracts of most domestic and wild animals as well as man. Its persistence in the environment is limited so its presence is often used as an indicator of faecal contamination of water or food (Synge, 2000). Although many strains of *Escherchia coli* are harmless inhabitant of the gastrointestinal tract, some can cause disease (Abd El-Khalek et al., 2001).

Some pathogenic *E.coli* strains produce cytotoxins similar to Shiga toxin of *Shigella dysenteria* 1 active on vero cells which are referred to as Shiga-like toxins (SLT) or vero toxins (VT). Vero cytotoxin-producing *E.coli* (VTEC) belong to many different O sera groups, but VTEC of serotype O157:H7 or O157:H- (non motile) are most frequently recognized as a cause of infection in man (Suthienkul et al., 1990 and Rozand, 1997).

Vero cytotoxins-producing *E.coli* O157 has been associated with a variety of diseases in humans, including bloody and non bloody

diarrhoea, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Capozzi, 1999). VTEC is the major cause of acute renal failure in children and is generally among the top three bacterial causes of diarrhoea in The United States (Acheson et al., 1996 and Slutsker et al., 1997).

Most human infections caused by VTEC result from the consumption of contaminated food, especially those of bovine origin, such as under cooked ground beef, unpasteurized cow's milk and by person-to-person contacts (De Boer and Heuvelink, 2000). Animals, particularly cattle and sheep, act as a reservoir of infection although the organism is rarely associated with animal disease (Hancock et al., 1994; Heuvelink et al., 1997; Rozand, 1997; and Abd El-Khalek et al., 2001).

The purpose of this study was to determine whether some meat products (luncheon and raw minced meat) and beef cattle in Assiut are potential sources of *E.coli*, particularly those of serogroup O157. We surveyed market foods and beef cattle for the prevalence of *E.coli* by using selective enrichment and plating media for detection of *E.coli* O157.

MATERIAL and METHODS

Sampling:

One hundred meat products samples (50 samples of each raw minced beef and luncheon) were collected from different restaurants and supermarkets at Assiut Governorate. Also one hundred faecal samples were taken from apparently healthy beef cattle immediately after slaughter. Each sample was wraped separately and aseptically in a sterile polyethylene bag then labeled and transferred in an insulated ice box to the laboratory with a minimum of delay for bacteriological examination as follow:

Twenty five grams from each meat products sample were blended in 225 ml of modified *E.coli* broth with novobiocin (mEc+n) (the constituents of the mEc were as follow: Tryptone (Difco) 20 g/L. Bile salts # 3 (Difco), 1.12 g/L, Lactose 5.0 g/L, K_2HPO_4 4.0 g/L, KH_2O_4 1.5 g/L, NaCl 5 g/L, beef extract 10 g/L and distilled water 1 liter. The pH was adjusted to 6.9 ± 0.1 before autoclaving at 121 °C for 15 min., after cooling sodium novobiocin was added to produce final

concentration of 20 mg/L (Okrend et al., 1990) using sterile blender at high speed for 3 min. In the same way a loopfull from each feacal sample was inoculated in mEc+n. The inoculated broth was incubated at 42°C for 24h. then loopfuls from the incubated broth were streaked onto predried surface of sorbitol MacConkey agar plate (SMAC) (Oxoid, CM 813) suplemented with cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L) (CT.SMAC) and incubated at 37°C for 24h. The selective CT-SMAC differentiates *E.coli* O157 from other *E.coli* strains by the absence of sorbitol fermentation (colourless colonies) (Heuvelink et al., 1996 and 1997).

The suspected colonies were identified morphologically by Gram's stain and biochemically confirmed as *E.coli* according to Verman and Evans (1991) by the conventional IMViC (indole, methyl red, voges proskauer and citrate) and triple sugar iron agar (TSI). The colourless colonies were identified as *E.coli* O157 according to Okrend et al. (1990), and De Boor and Heuvelink (2000) by: absence of B-glucuronidase on fluorescent lauryl sulphate tryptose broth (Mark Art Nr. 1.12588), production of indole at 44°C and don't ferment of raffinose and cellobiose. The isolates presumptively identified as *E.coli* O157 were confirmed serologically using commercial diagnostic *E.coli* O157 Latex agglutination (Oxoid DR 620 M) according to Rozand (1997). The other *E.coli* isolates were identified serologically by using polyvalent and monovalent *E.coli O* antisera prepared at Animal Health Research Institute, Dokki according to Sojki (1965) and Hassanin (1977), against standard *E.coli* serotypes which were obtained from the Veterinary Academy of Moscow.

RESULTS

The results are represented in Tables 1, 2 and 3.

DISCUSSION

Verotoxin-producing *E.coli* especially serotype O157 has been incriminated as a causative agent in single cases and outbreaks of several potentially fatal disease in humans such as HC, HUS and infantile diarrhoea. The cited sources include bovine-derived meat and milk,

faeces of cattle and contact with infected herds (Borie et al., 1997 and Chapman et al., 2000). To identify the reservoirs of *E.coli* O157 and routes of transmission to man, sensitive methods, including enrichment techniques are necessary as this pathogen has a very low infective dose and may only be present in small numbers in food, environmental and faecal samples (Chapman et al., 1997 and De Boer and Heuvelink, 2000). To overcome these problems, the samples were enriched in mEc+n at 42°C without shaking followed by spread plating onto CT-SMAC at 37°C for 24h. Bennett et al. (1995); Sanderson et al. (1995) and Heuvelink et al. (1997) reported that mEc+n and CT-SMAC were the most efficacious sensitive and cost effective media for selective enrichment and isolation of *E.coli* O157from minced beef and bovine feces. Enrichment of O157 STEC in mEc+n resulted significant larger number of cells than enrichment in other selective broths e.g. modified tryptone soy broth (Heuvelink et al. 1997). Szabo et al. (1986) reported that bile salt # 3 at 0.112% in the presence of 0.5% NaCl in mEc+n would allow the full recovery of *E. coli* O157 at 42°C while inhibiting non-enteric bacteria and the addition of 1% beef extract were doubled their growth (Okrned et al., 1990). In the same way Zadik et al. (1993) and Bennett et al. (1995) concluded that CT-SMAC increased the rate of isolation of O157 VTEC from inoculated minced beef and cattle rectal swabs. Tellurite inhibits the growth of *E.coli* strains other than O157 VTEC and other non-sorbitol fermenting species and cefixime inhibits the growth of *Proteus* spp. Moreover incubation conditions play effective role in isolation of *E.coli* O157. Incubation of mEc+n at 42°C without shaking effectively supress the other sorbitol non-fermenting organisms in ground beef such as *Hafnia alvei* while allowing good growth of O157 STEC (Blais et al., 1997).

According to the data summarized in Table (1), *E.coli* was detected in 30% (15/50), 46% (23/50) and 55% (55/100) from luncheon, minced meat and feces of beef cattle respectively. Nearly similar results were recorded by El-Shaboury et al. (1999) and Youssef et al. (1999). lower incidence of *E.coli* in minced meat and luncheon was reported by Ahmed (1992), while higher levels were obtained by Refaic and Nashed (1990). The variation in the results may be due to difference in manufacture practices, handling from producers to consumers and the effectiveness of hygienic measures applied during production.

In the present study, *E.coli* O157 was not detected in luncheon samples (Table 1). This may be due to the exposure to high temperature during processing and the highly acidic nature of this meat product. In an experimental study carried by Weagand et al. (1994), it was observed that *E.coli* O157 died rapidly in acid foods at room temperature, while survived for weeks at refrigeration temperature.

E.coli O157 was isolated from 4% (2/50) and 5% (5/100) of minced beef and fecal samples of beef cattle respectively (Table 1). Several investigators have detected *E.coli* O157 in meat products and feces of cattle. Doyle and Schoeni (1987) isolated *E.coli* O157:H7 from 4% of beef and 1.5% of poultry samples in Wisconsin, while 31% of beef samples from Calgary and Alberta, contained *E.coli* O157:H7. Abdul-Rauf et al. (1996) detected *E.coli* O157:H7 in 4% and 6% of Egyptian beef and chicken respectively. Chapman et al. (2000) detected low prevalence (1.1%) of *E.coli* O157 in ground beef.

The presence of *E.coli* O157 in meat could be attributed to the contamination from feces of infected animals as indicated by Suthienkul et al. (1990) who recorded that SLTEC were found in 11 to 84% of fecal matter of cattle before slaughter, from 8 to 28% of fresh beef specimen at slaughter houses using a DNA probe. Chapman et al. (1992) and Abdel Khalek et al. (2001) found that the prevalence of *E.coli* O157 in feces of cattle was 3% by using CT-SMAC media. Mechie et al. (1997) and Boric et al. (1997) reported an incidence of 14% and 34.5% respectively which seems to be high while Wells et al. (1991) failed to detect *E.coli* O157 in feces of milking cows.

This wide variation in carriage rate of cows to *E.coli* O157 may be explained in part by the variable efficiencies of the isolation protocols, the season and geographical area may also have an effect on prevalence figures (Chapman, 2000 and Synge, 2000).

Concerning to the biochemical reactions of *E.coli* serotype O157 (Table 2) was found to be negative for sorbitol fermentation, absence of B-glucouronidase, and positive for indole production at 44°C. Similar results were recorded by Okrend et al. (1990), Heuvelink et al. (1997) and De Boer and Heuvelink (2000). Rozand (1997) recorded that 93% of *E.coli* isolates of human origin ferment sorbitol within 24h and possess the enzyme B-glucuronidase, While *E.coli* O157 was reported as not fermenting sorbitol, and not producing B-glucuronidase. On the other

hand Gunzer et al. (1992) and Hayes et al. (1995) observed that some strains of *E.coli* O157 fermented sorbitol within 24h. Unlike most sorbitol and B-glucuronidase negative *E.coli* such as *Escherichia hermanii*, *E. coli* O157 do not ferment cellubiose and reffinose (De Boer and Heuvelink, 2000).

Serotypes of *E.coli* isolates rather than *E.coli* O157 of different studied samples revealed the following somatic serogroup, O119, O55, O117, O158, O114, O107, with different incidences (Table 3). Similar serotypes were obtained by Youssef et al. (1999) who recorded that the most common groups of *E.coli* in luncheon and minced meat were O55 and O119. Chapman et al. (1993) recorded another somatic *E.coli* serotypes O113, O168, O172 during examination of rectal swabs of dairy cattle.

In conclusion, *E.coli* O157 was detected in minced meat and faeces of beef cattle in our area which may represent a significant source of infection to man. Therefore stricted hygenic measures should be imposed at many levels ranging from farms, slaughter houses to home to minimize the risk of spread of *E.coli* O157 to animal and man.

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Table 1: Prevalence of total *E.coli* strains and *E.coli*; O157 in some meat products and faecal samples of apparently healthy beef cattle.

Samples	No. of Samples	*E.coli* strains		*E.coli* O157	
		No.	%	No.	%
A-Meat products					
1-Luncheon	50	15	30	0	0
2-Raw minced meat	50	23	46	2	4
Total	**100**	**38**	**38**	**2**	**2**
B-Faeces from beef cattle	**100**	**55**	**55**	**5**	**5**

Table 2: Relationship between biochemical and serological identification of *E.coli* O157.

Biochemical test	No. of isolates	No. of +ve isolates by latex agglutination	
		No.	%
Sorbitol ferementation (-Ve)	9	7	77.77
Absence of B-gluconidase (-Ve)	7	7	100
Production of indole at 44°C (+Ve)	8	7	87.5
Raffinose fermentation (-Ve)	8	7	87.5
Cellbiose fermentation (-Ve)	9	7	77.77

Table 3: Frequency distribution of *E.coli* serotypes other than *E.coli* O 157 isolated from the examined meat products and fecal samples

Samples	No. of Isolates	*E.coli* serotypes						
		O55	O107	O114	O117	O119	O158	untypable
Luncheon	15	3	2	1	1	2	3	3
Raw minced meat	21	3	-	-	4	6	2	6
Faeces from beef cattle	50	8	3	7	5	9	5	13