Journal of the Islamic University of Gaza, (Natural Sciences Series), Vol..12, No.1 PP.51-56, 2004

Occurrence of *Escherichia coli* O157 in Gaza Strip: A Preliminary Study

Fadel A. Sharif Medical technology Department of Islamic University of Gaza
Hashem Z. Arafa Public Health Lab. Sabha Central Lab, Gaza, Palestine.

مدى تواجد بكتريا إيشيريشيا كولاى O157 في قطاع غزة: دراسة أولية

ملخص: أجريت هذه الدراسة بغرض الكشف عن تواجد بكتريا إيشيريشيا كولاى O157 في أنواع مختلفة من العينات المنتقاة عشوائيا من جميع أنحاء قطاع غزة، خاصة وأنه لا يتوفر أي معلومات حول وبائية هذه البكتريا في القطاع شملت العينات التي تم فحصها: 190 عينة لحمة مفرومة، 300 عينة خضار، 152 عينة مياه شرب، 809 عينة براز من أشخاص مصابين بالإسهال، و 120عينة مياه عادمة. وكان تواجد بكتريا إيشيريشيا كولاى O157 في العينات كالتالي: 11.2% في عينات اللحمة المفرومة، 11.7% في الخضار، 9.9 % في عينات مياه الشرب، و 20.8 % في عينات المياه العادمة.

وأظهرت النتائج أن نسبة بكتريا إيشيريشيا كولاى O157 كانت أعلى في عينات البراز التي جمعت من فئة العمر 0-4 سنوات وكانت النسبة الكلية لوجود البكتريا في عينات البراز 10.6%.

Abstract: This study was carried out in order to investigate the occurrence of *E. coli* O157 in different types of samples collected randomly from all over the Gaza Strip. Data on prevalence of this emerging cause of food-borne illness in Gaza Strip are not available.
The samples tested in this study included: 190 minced meat samples, 300 vegetable samples, 152 drinking water samples, 809 diarrheal stool specimens and 120 sewage samples. The overall occurrence of *E. coli* O157 was as follows: 11.2% in minced meat, 11.7% in vegetable samples, 9.9% in drinking water and 20.8% in sewage samples.
E. coli O157 was more prevalent in diarrheal stools collected from the 0-4 years age group and its overall occurrence in stool specimens was 10.6%. These results implicate that this pathogen can not be ignored as a possible cause of diarrhea in Gaza Strip.
The heavy use of untreated organic fertilizers, lack of proper sewage disposal and poor hygiene practices are believed to be the main factors contributing to the occurrence of *E. coli* O157 in Gaza Strip.

Introduction

Toxigenic *E. coli* O157 is an emerging cause of food-borne illness. In humans infections with this serotype may cause mild diarrhea, bloody diarrhea (hemorrhagic colitis) and in children, infections with this pathogen may lead to the diarrhea-associated form of hemolytic uremic syndrome (HUS).[1]

The mechanism by which O157 strains cause disease are thought to be through the production of either or both of two phage-encoded toxins (verocytotoxin 1 and verocytotoxin 2) which cause the vascular endothelial damage observed in patients with hemorrhagic colitis and the HUS.[2]

Healthy domestic animals, in particular ruminants like cattle, sheep and goats are regarded as the principal reservoirs of *E. coli* O157.[3]

E. coli O157 can be transmitted to humans through direct or indirect contamination of food by fecal material. Undercooked beef and raw milk have most often been implicated in food-borne infections. In addition to consumption of contaminated foods, humans can become infected by direct transmission *E. coli* O157 from infected animals or by secondary spread from person to person.[4]

Reported estimates of the prevalence of *E. coli* O157 in many countries range from 0 to almost 10%. The isolation rate is greatly influenced by factors such as the target population, the sampling strategy and the screening method used. Furthermore, geographic and seasonal variations in prevalence may occur.[5]

E. coli O157 does not ferment sorbitol, and this fact is used in its isolation on sorbitol-containing bacteriological media.[3] MUG (4-methylumbelliferyl-β-D-glucuronide) assay used in conjunction with testing for sorbitol fermentation and agglutination in *E. coli* O157 antiserum is considered a useful screening test for toxigenic strains of *E. coli* O157.[6]

The objective of this study was to investigate the occurrence of *E. coli* O157 in Gaza Strip and determine the potential sources of infection by this strain, especially that, studies on this microorganism have never been undertaken in this area.

Materials and Methods

The present study was conducted over a period of two years (April, 1999 to March 2001). Samples were tested at the Public Health Lab for Food and Water, Gaza.

Sample Collection and preparation

Samples investigated in this study included: 190 minced meat samples, 300 vegetable samples, 152 drinking water samples, 809 diarrheal stool specimens and 120 sewage samples.

Minced meat samples (100 gram each) were collected in sterile plastic cups from Gaza Strip butchers, and were transferred to the lab in an icebox immediately after collection. 25 grams of each sample were mixed with 225 ml 0.1% peptone water and homogenized in a stomacher. Ten fold dilutions were then prepared using 0.1% peptone water as the diluent. Diluted samples were used within ten minutes of preparation.

Raw Vegetable samples (300 gram each) were collected from retail vegetable stores in sterile plastic bags and transported to the lab at ambient temperature. Parsley, cucumber, egg plant, green pepper, potato, marrow, tomato, French beans, Nalta jute (Molokhiye), white beat and okras were the main types of vegetables examined. Vegetable samples were treated as described for meat samples above.

Drinking water samples tested in this study were all coliform positive and were submitted to the lab for microbiological analysis from different regions of Gaza Strip. Moreover, most of these samples were from private wells.

Diarrheal stool specimens were collected on rectal swabs and transported to the lab in an icebox within one hour of collection. For the purpose of enrichment, each swab was placed in 5 ml peptone water supplemented with cefixime (0.05 mg/l), cefsulodin (10 mg/l) and vancomycin (8 mg/l). Fecal material was suspended in the medium by vortex mixing, and the mixture was incubated at 37ºC for 6 h.

Sewage samples were collected from Gaza Strip sewage outlets in sterile glass bottles and transported to the lab in an icebox within few hours of collection. One ml of each sample was enriched for *E. coli* O157 as described for the stool specimens above.

All samples were processed within 24 hours of collection.

Isolation and identification of *E. coli* O157

Suitable aliquots of the samples (with or without enrichment) were plated in duplicates on sorbitol-MacConkey (SMAC) agar (Oxoid). After 24 h incubation at 37ºC, sorbitol-negative (colorless) colonies (one or more colonies per plate) were selected for further testing. Isolates were confirmed as *E. coli* by biochemical testing using an API20E test strips (bio Merieux). Sorbitol-negative *E. coli* isolates were subcultured on violet red bile agar (Difco) containing 100 mg/l MUG. Plates were incubated at 37ºC for 18 h. MUG-negative colonies do not fluoresce upon exposure to short-wave ultraviolet light, E. coli O157 appear as violet colored colonies.

Sorbitol-negative, MUG-negative E. coli isolates were finally tested for the somatic O157 antigen, with slide agglutination, with E. coli O157 antiserum (Oxoid).

Results

The occurrence of E. coli O157 in the different samples investigated in this study is illustrated in Table 1.

Table 1. Occurrence of *E. coli* O157 in the tested samples.

Type of Sample	Number tested	Positive for *E. coli* O157	
		No.	%
Minced meat	190	21	11.2
Vegetables	300	35	11.7
Drinking water	152	15	9.9
Diarrheal stool	809	86	10.6
Sewage	120	24	20.0

In stool specimens, out of 607 specimens collected from the age group 0-4 years, 68 (8.4%) were positive for *E. coli* O157 while, only 18 (2.2%) of the 202 specimens collected from the age group > 4 years were positive (Table 2).

Table 2. Occurrence of *E. coli* O157 in stool specimens with respect to age

Age group	Stool specimens		Positive for *E. coli* O157	
	No.	%	No.	%
0-4 years	607	75	68	8.4
> 4 years	202	25	18	2.2
Total	809	100	86	10.6

Discussion

E. coli O157 is a global cause of infection, with documented cases throughout the US, Canada, the UK, Africa, Argentina, Chile, China, Japan and Saudi Arabia. Moreover, the organism has been isolated in numerous worldwide outbreaks of hemorrhagic colitis and HUS.[7]

In the present work, *E. coli* O157 was encountered in 20% of the sewage samples, and since untreated sewage is improperly disposed off in many regions of Gaza Strip, this practice entails a major risk of the spread of *E. coli* O157 into recreational waters and drinking water supplies. Isolating the pathogen from 9.9% of the drinking water samples, where most of the samples came from untreated private well water, supports this thesis.

E. coli O157 entering bathing and untreated drinking waters (e.g., private wells) is quite hazardous, especially because of the low infectious dose of this pathogen.[8]

Numerous studies have shown that healthy ruminants constitute a worldwide natural reservoir of *E. coli* O157, and that they serve as the primary source of the organism. Most of the ruminants in Gaza Strip are imported, through Israel, from various countries. Data on the level of *E. coli* O157 in those animals, however, are not available, and one of our future plans is to investigate the *E. coli* O157 fecal shedding rate in Gaza Strip ruminants.

The intensive use of untreated manure in agriculture and irrigating crops by sewage-contaminated water could be the main reasons behind the occurrence of *E. coli* O157 in 11.7% of the raw vegetables examined in the current study. Several investigators have isolated *E. coli* O157 from various raw vegetables, and they have shown that the contaminating source is the application of both animal (cattle slurry) and human (sewage sludge) wastes of fecal origin. [9,10]

Our results showed that the occurrence of *E. coli* O157 in minced meats was 11.2%. Published research has reported that the prevalence of the pathogen in meat animals, at the time of slaughter, varies from 0-60%.[11] Therefore,

and on the assumption that our animals contain the microorganism, the presence of *E. coli* O157 in minced meats must have occurred post-evisceration of carcasses. Consequently, the occurrence of the pathogen in minced meat samples suggests the ineffectiveness of the sanitary procedures followed by Gaza Strip butchers during the processing of meats.

A high level of *E. coli* O157 (10.6%) was encountered in the diarrheal stool specimens investigated in this study. Here we suggest that all diarrheal stools should be tested for this pathogen, especially because of the severe complications of *E. coli* O157 infection (e.g., HUS) and the adverse effect of antibiotic treatment for this organism.

Human infections by this pathogen are generally explained by consumption of undercooked meats, raw milk and contact with animals.[12] These are not, however, common practices in Gaza Strip, and we believe that the major sources of infection by *E. coli* O157 in our country are consumption of unwashed raw vegetables, drinking untreated water, and direct transmission from ill or asymptomatic carriers.

The higher occurrence of *E. coli* O157 infection in the age group 0-4 years as compared to older ages is in agreement with published data on this regard[13] and could be explained by the lack of awareness and proper hygiene practices among this age group.

From this preliminary study we conclude that the occurrence of *E. coli* O157 in Gaza Strip is relatively high. Amelioration of the threat of this foodborne pathogen should be part of an integrated program that will enhance food safety. Such a program should include the producer, the food processing steps, the distributors, retailers and the consumer.

References

1. Griffin PM and Tauxe RV. (1991). The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13:60-98.
2. Tesh VL and O'Brien AD (1991). The pathogenic mechanism of Shiga toxin and Shiga-like toxins. Mol. Microbiol. 5:1817-1822.
3. McDonough PL, Rossiter CA, Rebhun RB, Stehman SM, Lein DH, Shin SJ (2000). Prevalence of *Escherichia coli* O157:H7 from Cull dairy cows in New York State and comparison of culture methods used during preharvest food safety investigations. J. Clinical Microbiol. 38(1):318-322.
4. Armstrong GL, Hollingsworth J and Morris JG. (1996). Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a

new pathogen into food supply of the developed world. Epidemiol. Rev. 18: 29-51.

5. Heuvelink AE, Biggelear FL, Zwartkruis JT, Herbes RG, Huyben R, Nagelkerke N, Melchers WJ, Monnens LA and Boer E. (1998). Occurrence of verocytotoxin-producing *Escherichia coli* O157 on Dutch dairy farms. J.Clin. Microbiol. 36(12):3480-3487.

6. Anonymous (1994). *E. coli* O157:H7: Procedure for isolation and identification from stool specimens. National Center for Infectious Disease, Centers for Disease Control and Prevention MS C-09 1600 Clifton Rd Atlanta, GA 30 333.

7. Kuntz TB, Kuntz ST (1999). Enterohemorrhagic *E. coli* infections. Prime Care Updates Ob/Gyns 6(6):192-196.

8. Willshaw, GA, Thirlwell J, Jones AS, Parry S, Salmon RL, Hickey M (1994). Verocytotoxin-producing Escherichia coli O157 in beef burgers linked to an outbreak of diarrhea, hemolytic colitis and hemolytic uremic syndrome in Britain. Lett. Appl. Microbiol. 19: 304-307.

9. Fujisawa, T and Mori M. (1992). Species of coliform bacteria isolated from raw vegetables. J. Antibact. Antifungal Agents, Jpn 20:251-253.

10. Ogden ID, Fenlon DR, Andrew VJA, Lewis D (2001). The fate of *Escherichia coli* O157 in soil and its potential to contaminate drinking water. International J, Food Microbiol 66: 111-117.

11. Elder RO, Keen JE, Siragusa GR, Gallagher BGA, Koohmaraie M, Leagreid WW. (2000). Correlation of enterohemorrhagic of *Escherichia coli* serotype O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. USA 97(7):2999-3003.

12. Doyle MP. (1991). *Escherichia coli* O157:H7 and its significance in foods. Int. J. Food Microbiol. 12:289-302.

13. Verweyen HM, Karch H, Allerberger F, Zimmerhacki LB. (1999). Enterohemorrhagic Escherichia coli (EHEC) in pediatric hemolytic-uremic syndrome: a prospective study in Germany and Austria. Infection 27(6):341-347.