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OCCURRENCE OF *E.COLI* O157:H7 IN APPARENTLY HEALTHY DAIRY CATTLE AND RETAIL MILK.

(With 3 Tables and 1 Figures)

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مدى تواجد ميكروب الايشريشيا كولاى O157:H7 في الأبقار الحلو ب السليمة ظاهريا و الألبان المتداولة في الأسواق

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إن المخاطر التي يتعرض لها الإنسان بعدوى الاشريشيا كولاى *O157:H7* نتيجة لتناول ألبان الأبقار الخام لا تزال في ازدياد و تظهر عدة على هيئة حالات فرديه أو في صورة اوبئة. و حيث أن لبن الأبقار يعتبر من المصادر الهامة لنقل هذا الميكروب، لذلك فقد أجريت هذه الدراسة لتحديد دور الأبقار الحلوب السليمة ظاهريا كمصدر للعدوى بهذا الميكروب. وقد تم جمع عدد ١١٠ عينة بواقع ٨٠ عينة من الأبقار السليمة ظاهريا (٤٠ عينة ألبان و ٤٠ عينة براز) هذا بالإضافة إلى عدد ٣٠ عينة من الألبان المعروضة للبيع في مناطق مختلفة من محافظة أسيوط و ذلك في الفترة من شهر يونيو إلى سبتمبر ٢٠٠٢. و قد أسفرت النتائج عن وجود هذا الميكروب في العينات المختبرة بنسبة ١٢,٧٣% . و قد تم عزله من 12 (30%) ، 1 (2.5%) و 1 (3.33%) من عينات البراز و الألبان و الألبان المعروضة للبيع على التوالي. و قد تم عزل الميكروب من عينات البراز فقط بنسبة ٢٧,٥% و عينات الألبان و البراز من نفس الحيوان بنسبة ٢,٥%. و قد تبين أن الأبقار السليمة ظاهريا تلعب دور هام كمصدر لميكروب الايشريشيا كولاى *O157:H7* مما يزيد من احتمال تلوث الألبان و الذى يشكل خطورة على صحة الإنسان.

SUMMARY

Risk of acquiring verocytotoxin *E.coli* infections through ingestion of raw cow's milk continues to increase causing either sporadic cases or outbreaks. During June-September 2002, one hundred and ten samples were screened for the presence of verocytotoxin-producing *E.coli* (VTEC). Both milk samples (40) and fecal swabs (40) were collected from the same apparently healthy cows in dairy farms, moreover thirty

samples of retail milk marketed in Assiut province were collected. Verocytoxin-producing *E.coli* was isolated from 14 (12.73%) of 110 samples after enrichment in mTSB (modified tryptic soya broth) supplemented with novobiocin, plating onto SMAC (sorbitol MacConkey agar) and then were identified using latex agglutination test. *E.coli* O157:H7 strains were confirmed by using polymerase chain reaction to detect hyl A gene (target of *E.coli* O157:H7). Verocytotoxin producing *E. coli* was recovered from 12 (30%), 1 (2.5%) and 1 (3.33%) of fecal swabs, milk samples and retail milk, respectively. *E. coli* 0157:H7 was isolated from 27.5% fecal swabs only and 2.5% of both milk and feces of the same animal, however we could not isolate the organism from the milk samples only. It was concluded that apparently healthy dairy cows may act as reservoirs of verocytotoxin-producing *E.coli* with fecal shedding representing a risk factor of milk contamination which pose a public health hazard.

Key words: *E.coli* O157 : H_7 – *Dairy cattle – Milk – PCR..*

INTRODUCTION

E.coli O157:H7, is a new emerging, clonally distinct form of *Escherichia coli* was first identified as a significant food-borne zoonotic pathogen (Riley *et al.*, 1983). Verocytotoxin-producing *E.coli O157:H7* (VTEC O157) has been emerged as a major food-borne pathogen which has the most sever effect on young children and the elderly (Griffin, 1995 and Feng *et al.*, 1998).

Although over 150 different OH serotypes of verocytotoxin producing *E.coli* (VETC) have been associated with human illness, the vast majority of reported outbreaks and sporadic cases of VTEC infections in humans have been associated with serotype *O157:H7* causing a range of symptoms from mild non bloody diarrhoea to haemorrhagic colitis, life threatening haemolytic uremic syndrome and thrombocytopenic purpura through the production of the shiga-like toxins and other probable virulence factors. (Riley *et al.*, 1983; Slutsker *et al.*, 1997 and Nelson *et al.*, 1998).

Epidemiological investigations have revealed that dairy cattle, especially young animals are the principal reservoirs of *E.coli O157:H7* which can be excreted by healthy carriers and diarrhoeic cattle (Hancock *et al.*, 1994; Zhao *et al.*, 1995; Porter *et al.*, 1997 and Clarke et al, 1998). Most reported human cases are associated with contaminated

food of bovine origin especially raw milk and milk products (Griffin, 1995).

This study aimed to determine the existence of *E.coli O157:H7* in feces and milk of apparently healthy cows as well as in the retail milk. At the meantime, we applied the polymerase chain reaction on the isolated strains to detect hly A target gene of *E.coli O157:H7*.

MATERIALS and METHODS

1. Samples collection:

One hundred and ten samples including individual cow's milk (40) and fecal swabs (40) of apparently healthy cows from dairy farms as well as retail milk (30) were collected from Assiut Province during the period from June to September 2002. Samples were transferred to the laboratory in an icebox for bacteriological examination.

2. Enrichment Technique:

Milk and fecal samples were enriched in modified Tryptic Soya Broth (mTSB) supplemented with novobiocin (20 mg/liter). The inoculated broth was incubated at 37 °C for 24 hours (De Boor and Heuvelink, 2000).

3- Isolation on Sorbitol MacConkey agar:

Loopful from the incubated broth was streaked onto Sorbitol MacConkey agar plates and incubated at 37 °C for 24 hours (De Boor and Heuvelink, 2000).

4- Identification of E.coli O157:H7:

4.1- Identification of E.coli :

Non sorbitol fermenter colonies were identified morphologically by Gram's stain and biochemically as *E.coli* according to Varnam and Evans, (1991) by the conventional IMViC(indole, methyl red, Voges Proskauer and Citrate utilization) and Triple sugar iron agar.

4.2- Latex agglutination test:

A latex agglutination test (*E.coli O157*, Oxoid diagnostic reagents 620 M) for identification of *E.coli* serogroup O157 had been done on 18 isolates of the *E.coli* culture. The Oxoid *E. coli O157* latex was demonstrated by slide agglutination of *E.coli* strains possessing the O157 serogroup antigen (Borcyzk *et al.*, 1987, Krishnan *et al.*, 1987 and Vernozy-Rozand, 1997). For each test sample, bacterial colonies were screened by a rapid latex test to ascertain their O157 infection status. If the test was positive, the suspected bacterial colony was subcultured

onto trypticase soya broth and incubated overnight at 37 °C for DNA extraction and amplification.

4.3- Detection of the E.coli O157:H7:

A. DNA extraction:

Nucleic acid was extracted from the trypticase soya broth culture as described by Pitcher *et al.* (1989), Boom *et al.* (1990) and Fathi *et al.* (2002) by using the chaotropic agent Guanidium thiocynate (GuSCN).

B. PCR procedure:

Amplification was performed in a total volume of 25 µl of DNA extracts, 25 pmol of each primer (*E.coli* O157-3 & O157-4) according to Wang *et al.*, 1997), and PCR mixture (Ready To. Go™ PCR Beads, Amersham Pharmacia Biotech, Austria). The PCR was conducted in a Biometra thermal Cycler (Biometra-Germany). The amplification condition was one cycle of 94 °C for 15s, then 35 cycles of 94 °C for 3s, 50 °C for 10s and 74°C for 35s, and finaily one cycle of 74 °C for 2 min. and 45 °C for 2s. The PCR products (10-15 µl of each) were separated by electrophoresis in 2% agarose gels containing ethidium bromide (1 µg/ml), and visualized under UV illumination.

RESULTS

Table 1: Existence of Non-sorbitol fermenters *E.coli* in Dairy cows

Source of samples	No. of samples	NSF *E.coli* Strains	
		No.	%
Feces	40	15	37.5
Milk	40	2	5
Retail milk	30	1	3.33
Total	**110**	**18**	**16.36**

*. NSF = Non Sorbitol fermenter

Table 2: Surveillance of verocytotoxin *E.coli* in Dairy cows.

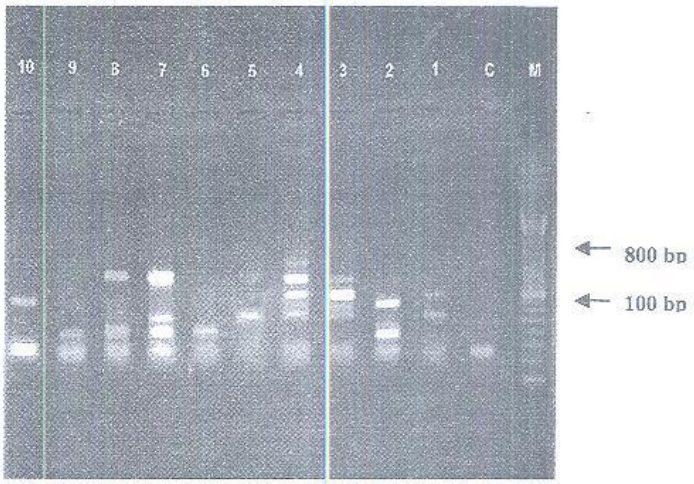
Source of samples	No. of samples	*E.coli* O157:H7	
	No.	No.	%
Feces	40	12	30
Milk	40	1	2.5
Retail milk	30	1	3.33
Total	**110**	**14**	**12.73**

Table 3: Occurrence of verocytotoxin *E.coli* in feces and milk of Dairy cows.

No. of isolated *E.coli* O157:H7	Feces only		Milk only		Milk & Feces	
	No.	%	No.	%	No.	%
	11	27.5	-	-	1	2.5

Fig 1: PCR examination of *E.coli* O157:H7

E.coli O157:H7 hly A gene O157-3, GTAGGGAAGCGAACAGAG and O157 4, AAGCTCCGTGTGCCTGAA (Amersham Pharmacia Biotech, Austria). The size of amplicon product (bp) ranged from 300-600 in this study.



PCR amplification of DNA extracted from *E.coli* O157 samples in a 2% agarose gel. Lane (M) is 100 bp DNA size marker (100 Base-Pair Ladder, Amersham Pharmacia Biotech); Lane (C) is PCR assay reagent control, Lane 1 to 10 are the positive isolates for *E.coli* O157 H7 of the 14 positive samples.

DISCUSSION

Dairy cattle have been implicated as a major reservoir of verocytotoxin *Escherichia coli* (Wells *et al.*, 1991). It is noteworthy that *E.coli* O157:H7 are shedded in feces of either healthy carriers or diarrhoeic cattle (Clarke *et al.*, 1998). Unlike typical *E.coli*, isolates of *E.coli O157:H7* do not ferment sorbitol, therefore this criteria is commonly used for selective isolation of *E.coli* O157:H7 (Tarr *et al.*, 1997). It was recently documented that for better isolation of *E.coli* O157:H7 from milk and feces, enrichment in mTSB (modified tryptone soya broth) supplemented with novobiocin is recommended, then plating onto SMAC (Sorbitol MacConkey agar) (De Boor and Heuvelink, 2000). Furthermore, Johnson *et al.* (1995) suggested that mTSB plus novobiocin enhances the availability of O157 antigen.

Non sorbitol fermenter (NSF) *E.coli* strains were isolated from 15 (37.5%) of 40 cow's feces, 2 (5%) of cow's milk and 1 (3.33%) of 30 retail milk samples as illustrated in Table 1. *E.coli* strains were typed as *E.coli O157* by using a latex agglutination test (*E.coli O157*, Oxoid diagnostic reagents 620 M). *E.coli O157* were confirmed as *E.coli O157:H7* by using polymerase chain reaction to detect hyl A gene (target of *E.coli O157:H7).* In this paper we used Guanidium thiocynate (GuSCN) in the purification and detection of DNA because of its potency to lyse cells combined with its potency to inactivate nuclease (Thompson & Gillespie, 1987 and Boom *et al.*, 1990).

Polymerase Chain Reaction (PCR) techniques could be developed as a routine procedure for food borne pathogen detection (Wang *et al.*, 1997). In our study, fourteen isolates were positive by using PCR technique (Fig. 1). This result agreed with that mentioned by Wang *et al.* (1997) who recorded that multiple DNA bands might be observed in the PCR detection from pure culture. The obtained results were comparable to that reported by Holland *et al.* (2000) who used the multiplex PCR for detection of *E.coli O157:H7*. McDonough *et al.* (2000), justify the using PCR techniques as an important tool for the detection of shiga-like toxins producing *E.coli*.

Verocytotoxin *E.coli* (*E.coli O157:H7*) was recovered from 12 (30%) of the examined cow's feces (Table 2). Geue et al (2002) reported variable rates of *E.coli O157:H7* recovery in feces of cows ranged from 29-82%. However lower rates of verocytotoxin *E.coli* prevalence in cow's feces were detected by Chapman *et al.* (1993b); Chapman *et al.* (1997); Mechie *et al.* (1997); Dutta *et al.*(2000); Abdel-Khalek *et al.*

(2001); Lahti *et al.* (2001) and Nielson *et al.* (2002) who estimated a prevalence rate of 9.4%, 16.1%, 14%, 1.7%, 3%, 1.31% and 21%, respectively. On the contrary, Wells *et al.* (1991) failed to isolate *E.coli O157:H7* from feces of milking cows. These differences in the estimated prevalence rates could be attributed to epidemiological variation in the different geographical localization.

E.coli O157:H7 was detected in 1 (2.5%) of the examined farm cow's milk (Table 2). Higher prevalence rate (10%) was estimated in individual cow's milk by Chapman *et al.* (1993 a), while Abdel-Khalek *et al.* (2001) reported a slightly lower prevalence with a rate of 2%. The variation in the detected prevalence rates in milk may be due to the difference in sources of contamination. It was reported that the risk of acquiring *E.coli O157:H7* infections through ingestion of raw milk continues to increase (Ryser, 1998) causing either sporadic cases or outbreaks of VTEC infection (Griffin and Tauxe, 1991 and CDC, 1993). We could able to recover *E.coli O157:H7* from 1 (3.33%) of the examined retail milk samples (Table 2). Our results are nearly similar to those detected by Abdel-Khalek *et al.* (2001) who estimated a prevalence rate of 3%. Results in Table 3 declared that verocytotoxin *E.coli* could be recovered from 11 (27.5%) of fecal samples only. The high percent of recovery of verocytotoxin *E.coli* in the examined feces is of particular concern indicating a major risk of milk contamination probability that pose a health hazard. Although not currently recognized as a cause of mastitis, presence of verocytotoxin *E.coli* in milk usually indicates contamination from feces of the infected or carrier animals (D'Aoust, 1989). This criteria explains both the ability to isolate the organism from both milk and feces of the same animal (with a rate of 2.5%) as well as the inability to recover verocytotoxin *E.coli* from milk samples only, as illustrated in Table 3.

It is concluded that dairy cows may act as carriers of verocytotoxin *E.coli* with fecal shedding which act as a major source of milk contamination. Moreover, it has been isolated from both farm raw milk and retail milk which act as a major risk factor for consumers that can lead to life threatening sequelae especially in young children and the elderly (Feng *et al.*, 1998). Indeed, this situation needs to be reviewed regularly and addressed if circumstantial evidence indicates that animals and animal products may be a significant reservoir of human *E.coli O157:H7* infections (Thorns, 2000).

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