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A study on enteropathogenic *Escherichia coli* isolated from domestic Iranian soft cheese

Ladan Mansuri Najand[1]*, Reza Ghanbarpour[2]

[1]*Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran*

[2]*Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran*

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ABSTRACT

The aim of the present study was to determine the prevalence of contamination of the domestic Iranian soft cheese with EPEC serogroupes in south-eastern Iran-Kerman. Seventy-seven random samples of domestic Iranian soft cheese were collected from different groceries and markets in sterile conditions, cultured in selective media and examined for biochemical tests. Among the 77 samples, *E coli* was isolated from 76 (98.70%) of them and 15 (19.48%) of the isolates were EPEC. Eight EPEC identified serogroups were included: O26(I), O86(II), O114(IV), O142 (IV and I), O119(II), O128(III), and O127(II). All these types are pathogens. O127 was the most prevalent serogroup, followed by O128 and O119. The occurrence of a high proportion of *E coli* in our cheese samples may be due to lack of proper sanitation and absence of pasteurization of milk used for cheese making. Therefore, stringent hygienic measures must be followed and pasteurization of milk should be imposed to prevent contamination of cheese with coliforms, thus avoiding additional outbreaks of food-borne illness caused by *E coli*.

Key words: *Escherichia coli*, cheese, serotyping

Introduction

E. coli is responsible for several outbreaks of diarrhoea in children and adults after ingestion of contaminated milk and dairy products. Different studies showed that 1 - 5% of food-borne infections were related to consumption of milk and dairy products, that 53% of cases of food-borne infections caused by contaminated cheese and that enteropathogenic *E. coli* (EPEC) is the causative agent of 18.33% of these cases (SCHRADE and YAGER, 2001).

* Contact address:
Assist. Prof. Dr. Ladan Mansuri Najand, Department of Food hygiene, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, P.O. Box 7616914111, Kerman, Iran, Phone and Fax: +98 341 3222 047; E-mail: mansouri39@yahoo.com

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EPEC strains have been implicated in food-borne human illnesses, especially as an important agent of infantile diarrhoea in developing countries (SILVA et al., 2001). EPEC strains are genetically mutative and cause different types of diarrhoea in patients. A combination of these strains with entrohemorrhagic *E. coli* increases their ability to create systematic infections in humans (DLSVIK et al., 1991). The pathogenecity of EPEC strains is not known completely but most of them produce Vero toxins which are different from the enterotoxigenic *E. coli* (ETEC) toxins (CAMPOS et al., 1994; CROSSLY et al., 1955). Some EPEC strains are attached to intestine epithelium (CAMPOS et al., 1994). Different strains of EPEC cause a different level of infection, and it has been shown that these strains are continuously changing and their genome becomes similar to hemorrhagic strains (CAMPOS et al., 1994).

Coliforms could be found in cheeses and are used as a hygienic indicator for such products. The presence of coliforms in cheese and their relation to entropathogenic *E. coli* in soft cheeses has received considerable attention in previous studies (SCHRADE and YAGER, 2001; MARIER et al., 1973). The relation between pathogenicity and different serotypes of *E. coli* has been suggested and proved (CROSSLY et al., 1995).

Contamination of dairy products by EPEC strains has been investigated and O126, O128, O25 and O125 were isolated (ABBAR, 1988; AHMED et al., 1988). Cheese has been known as an important animal origin product and in most countries domestic and traditional produced cheese are in demand. Despite improvements in the cheese making industry in Iran, domestic cheese is still very popular. Domestic cheeses are usually produced from raw milk with insufficient hygienic quality. Moreover, production and transportation of these products are carried out in poor hygienic conditions.

The present study was undertaken to record the serotypes and prevalence of contamination of domestic Iranian soft cheese with EPEC strains in south-eastern Iran-Kerman.

Materials and methods

In this study, 77 samples of domestic soft cheese were collected randomly from different groceries and markets in Kerman. On each occasion 100-500 g of cheese were collected in sterile conditions and transported in an ice bag to the food microbiology laboratory of the Faculty of Veterinary Medicine and were examined immediately. Sampling methods and bacteriological examinations were carried out according to dairy products standard methods (DERZANT and SPLITTOSER, 1992; MARVIN, 1976; ARAUJO et al., 2002; AHMED et al., 1988). Five g of samples were prepared and 45 mL Brain Heart Infusion Broth (BHI) (Biolife Laboratories, Italy) added, and incubated in 35 °C for 2 h. After incubation they were cultured in MacConkey Agar (MC) (Biolife Laboratories, Italy) and Levine's Eosin Methylene Blue Agar (LEMB) (Biolife Laboratories, Italy). The remainder of the BHI media

was added to 50 mL Tryptic Soy Broth (TSB) (Biolife Laboratories, Italy) and incubated in 45 °C for 18 h. and cultured again in MC and LEMB Agar. The selected colonies from MC and LEMB were chosen and examined for biochemical properties, such as production of urease, indol, citratase, H2S and etc. *E. coli* isolates were identified as described by QUINN et al. (1994). In general, 625 colonies were obtained and examined separately. Serotyping of *E. coli* isolates was carried out at the Pasteur Institute, Tehran, Iran.

Results

Among the 625 colonies examined by biochemical tests, the bacteria of 219 colonies were detected as *E. coli*. According to the results 219 *E. coli* isolates belonged to76 (98.70%) of cheese samples. Among the 76 contaminated cheese samples, eight EPEC serogroups were isolated from 15 (19.48%) of the samples. EPEC serogroups included O26(I), O86(II), O114(IV), O142(IV and I), O119(II), O127(II), and O128(III). The present observation indicated that O127 was the most prevalent serogroup, followed by O128 and O119. The details of results of biochemical and serological tests are presented in Table 1.

Table 1. Details of biochemical tests and number and percentage of isolated serogroups

Serogroup	Number and percentage	Citrate	Indole	Motility	MR	VP
O26	1 (6.66%)	-	+	+	+	-
O86(II)	1 (6.66%)	-	-	+	+	-
O114(IV)	1 (6.66%)	-	+	+	+	-
O119(II)	2 (13.33%)	-	+	+	+	-
O127(II)	5 (33.33%)	-	+	-	+	-
O128(III)	3 (20%)	-	-	+	+	-
O142(I)	1 (6.66%)	-	+	+	+	-
O142(IV)	1 (6.66%)	-	+	+	+	-

Discussion

In this study *E coli* was isolated from 98.70% of samples, and 19.48% of isolates belonged to the EPEC serogroup. Recent studies in other countries indicate similar results. ARAUJO et al. (2002) detected high levels of faecal contamination in 95.5% of cheese samples in Brazil, and EPEC was isolated from 21.1% of the samples. In Egypt, 60 Karish cheese samples were examined and *E. coli* was isolated from 75% of the samples. Similarly, ABBAR and KADDAR (1991) reported that 40.5% of cheese samples in Iraq were contaminated with EPEC strains.

In the present study we detected 8 different EPEC serogroups in Iranian soft cheese and that O127 was the most prevalent serogroup. Recent studies in other parts of world indicate different prevalent EPEC serogroups in soft cheeses. In Brazil, O127 was the most frequently found serogroup, followed by O55 and O26 (ARAUJO et al., 2002). AHMED et al. (1988) showed that most prevalent serogroups in Egypt are O111, O126, O128, O26, O25 and O125. In Iraq, four EPEC serogroups, including O111, O86, O125 and O119, are commonly isolated from cheese samples (ABBAR, 1988). In this study, 13.33% of EPEC serogroups were detected as O119 serogroup. Similarly to our results, ABBAR (1988) reported that O119 is one of the prevalent EPEC serogroups in cheese. In Australia, O119 serogroup was isolated from 161 patients with diarrhoea symptoms due to consumption of soft cheese (CROSSLY et al., 1995).

In another study in the west of Iran, *E. coli* was isolated from 51% of domestic Iranian soft cheese samples (SHIDFAR et al., 2004). It appears that the rate of contamination of soft cheese with *E. coli* varies in different parts of Iran.

According to the results of this study, 19.48% of isolated *E. coli* strains from domestic soft cheese belong to EPEC strains.

In conclusion, isolation of EPEC serogroups from domestic soft cheese represents a potential, as well as an indication, of the presence of other enteropathogens. Although recent studies on virulence factors indicate that not all EPEC strains are able to attaching/effacing lesion, it is however believed that high prevalence of contamination with EPEC strains increases the risk of infection for children, due to consumption of domestic soft cheese. It seems that further epidemiological investigations are needed to reveal the importance of contamination in domestic soft cheese in this area of Iran.

The occurrence of a high proportion of *E. coli* in our cheese samples may be due to lack of proper sanitation and absence of pasteurization of milk used for cheese making. Therefore, stringent hygienic measures must be followed and pasteurization of milk should be imposed to prevent contamination of cheese with coliforms. Otherwise, these traditional cheeses should be collected from the market.

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SAŽETAK

Cilj istraživanja bio je utvrditi prevalenciju kontaminacije domaćega iranskoga mekanoga sira enteropatogenim serološkim skupinama *E. coli* (EPEC) u jugoistočnom Iranu (Kermanu). Ukupno 77 slučajno odabranih uzoraka domaćega iranskoga mekanoga sira prikupljeno je pod sterilnim uvjetima iz različih trgovina i tržnica. Uzorci su bili nacijepljeni na selektivne hranjive podloge i pretraženi biokemijskim testovima. *E. coli* bila je izdvojena iz 76 (98,70%) od 77 pretraženih uzoraka od čega je 15 (19,48%) izolata bilo enteropatogeno. Identificirano je bilo osam EPEC seroloških skupina: O26(I), O86(II), O114(IV), O142(IV i I), O119 (II), O128(III) i O127(II). Svi su navedeni tipovi patogeni. Najčešće je bila izdvojena serološka skupina O127, a slijedile su O128 i O119. Visoki udio pojavnosti *E. coli* u uzorcima pretraženih sireva može se pripisati nedostatnoj sanitaciji i izostanku pasterizacije mlijeka koja se primjenjuje pri izradu sira. Zbog toga se moraju primjenjivati strože higijenske mjere te uvesti pasterizacija mlijeka kako bi se spriječilo zagađenje koliformnim uzročnicima. Na taj način izbjegle bi se zaraze uzrokovane hranom zagađenom bakterijom *E. coli*.

Ključne riječi: *Escherichia coli*, sir, serotipizacija, enteropatogeni sojevi