Using Culture and PCR Technique for Detection of *Escherichia coli* O157:H7 in Duhok Cheese

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ABSTRACT: This study was aimed to investigate the presence of *Escherichia coli* O157:H7 in Duhok cheese by culture and Polymerase Chain Reaction (PCR) technique. The cheese samples (n:120) were purchased from different supermarkets and shops from Zakho, Akre, Amedy and Duhok cities during September 2011 to February 2012.

X-gal positive, MUG negative, indole positive colonies were found as 10% (12/120) of samples. These colonies were confirmed by biochemical tests (API E 20 test, latex agglutination tests) and results showed that 7 (5.83%) of *E. coli* isolates from cheese were positive for O157:H7. These isolates were also confirmed by PCR assay using specific primers for *rfb*O157 and *flic*H7. The numbers of positive isolates for *rfb*O157 and *flic*H7 were 3.33 and 0.83%, respectively. The PCR results confirmed as 0.83% of samples were *E. coli* O157:H7.
Key words: Duhok cheese, *Escherichia coli* O157:H7, Cultural technique, PCR technique

Kültür ve PZR Yöntemi ile Duhok Peynirinde *Escherichia coli* O157:H7'nin Araştırılması

ÖZET: Bu çalışmada Duhok peynirinde of *E. coli* O157:H7'nin varlığı kültür ve Polimeraz Zincir Reaksiyonu tekniği (PZR) ile araştırılmıştır. Peynir örnekleri (n:120) Zakho, Akre, Amedy ve Duhok şehirlerinden Eylül 2011-Şubat 2012 tarihleri arasında yerel market ve bakkallardan temin edilmiştir.

X-gal pozitif, MUG negatif, indol pozitif olan %10 (12/120) oranında şüpheli koloni tespit edilmiştir. Şüpheli kolonilere biyokimyasal testler uygulanmış (API E 20 ve latex aglütinasyon testi) ve 7 (%5,83) örnekte *E. coli* O157:H7 tespit edilmiştir. Bu izolatlara*rfb*O157 ve *flic*H7 primerleri kullanılarak PCR yöntemi ile doğrulama işlemi uygulanmıştır. İzolatlar %3,33 oranında O157 ve 0,83 oranında H7 antijeni pozitif olarak tespit edilmiştir. PCR analizlerinin sonunda örneklerin %0,83'ünün *E. coli* O157:H7 olduğu gözlenmiştir.
Anahtar Kelimeler: Duhok Peyniri, *Escherichia coli* O157:H7, Kültür tekniği, PZR tekniği

INTRODUCTION

Enterohaemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 is a foodborne pathogen. The serotype O157:H7 is predominant serotype among enterohaemorrhagic *E. coli*, and associated with foodborne outbreaks (Ferens and Hovde, 2011). This bacteria causes diarrhea, bloody diarrhea, life-threatening post diarrheal disorder (hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Karmali, 1989; Andreoli et al., 2002).

Raw milk is considered as a good medium for growth of many different pathogenic bacteria included *E. coli* O157:H7. Since healthy domestic animals, especially cattle, sheep and goats, can harbor STEC and O157:H7 in their feces (Blanco et al., 2003; Beutin et al., 2004). Transmission occurs through consumption of undercooked meat, unpasteurized dairy products and vegetables, or water contaminated by feces of carriers. Also, person-to-person transmission has also been documented (Beutin et al., 2004).

Cheese is an important milk product produced in almost every part of the world. It is widely consumed by the majority of people. Different studies showed that 1-5% of foodborne infections were related to consumption of milk and dairy products, that 53% of cases of foodborne 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). Contaminated unpasteurized dairy products such as raw milk and raw-milk cheese have been incriminated in recent foodborne STEC outbreaks (Honish et al., 2005; CDC, 2007). The ability of survival of the pathogen in raw goat milk lactic (soft) cheeses, aged cheddar cheese made from unpasteurized milk (Schlesseret al., 2006), feta cheese and yogurt (Morgan et al., 1993; Govariset al., 2002) were documented.

This study was aimed to investigate the incidence of *E. coli* O157:H7 in local Duhok cheese collected from Northern Iraq /Duhok Province under Duhok, Amedy, Zakho, Akrecities. Local cheese is a conventional soft cheese which is manufactured from unsterilized raw milk in farmer houses and frequently consumed in Northern Iraq.

MATERIAL and METHODS

The samples of local cheese (n:120) were obtained from different supermarkets and shops in Northern Iraq/Duhok Province under the following districts; Duhok city center (n:40), Amedy (n:20), Zakho (n:28)

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and Akre (n:32). The samples were collected from September 2011 to February 2012. The samples were brought to the laboratory immediately in ice pack.

Microbiological Analyses

A total of 25 g sample was enriched in 225 mL Butterfield's phosphate buffer at 37°C ± 1°C for 18-24 h. The enriched culture was plated on TC-SMAC (BD, BBL, 222226) and R&F *E. coli* O157:H7 agar (Merck, 1.04036.0500) and incubated at 37°C ± 1°C for 18-24 h. On TC-SMAC typical O157:H7 colonies are colorless or neutral/gray with a smoky center and 1-2 mm in diameter. Sorbitol-fermenting bacteria such as most *E. coli* appear as pink to red colonies. On *E. coli* O157:H7 agar, *E. coli* O157H7 colonies are black to blue-black colonies. *E. coli* O157:H7 (ATCC 43895) was used as a control. Suspected typical colonies were tested for O157 antigen by latex agglutination (RIM *E. coli* O157:H7 Latex Test (Remel, Lenexa, KS, 800-255-6730). Also the suspected colonies were tested for purity with TSAYE. Coli Complete (CC) disc (BioControl, Bellevue, WA) was placed a in the heaviest streak area on the TSAYE plate and incubated at 37°C ± 1°C for 18-24 h. Blue color on and around the disc (indicative for coliforms) and blue fluorescence around the disc was checked under long wave UV (365 nm) light (indicative of *E. coli*). X-gal (+), MUG (-), indole (+) colonies were assumed as *E. coli* O157:H7 (Feng et al., 2011). Then the colonies were re-purified on Blood agar for API 20E test for confirmation (Stampi et al., 2004) and polymerase chain reaction (PCR) assay using the O-antigen-encoding region of *rfb*O157 and *flic*H7 generic primers as described before (Gannon et al., 1997; Paton and Paton, 1998).

DNA Extraction

DNA of *E. coli* that isolated from cultural method was extracted from few colonies grown on Mac Conkey Agar (Merck) plates by boiling method. One loopful of *E. coli* from agar plates was suspended in 100 µl of sterile deionized water in an eppendorf tube and a bacterial suspension was made by using vortex. The bacterial suspension was boiled at 100ºC for 5 minutes and centrifuged at 10,000 x g for 2 min. The supernatant was used as DNA template for PCR (Tobias and Vutukuru, 2012).

PCR Preparation

Allthe PCR reactions were performed in 20 µl final volume containing 0.5 µl of thetemplate DNA, 10 µl of ReddyMix (containing KAPA2G FastHotStart DNA Polymerase, buffer, 0.2 mM of each dNTP, 1.5 mM of $MgCl_2$, at the final concentration of 1.5 mM) (Techtum, Sweden). Additional $MgCl_2$ to the final concentration of 2 mM, and each of the primers (MWG, Germany) to the final concentration of 10 µM were added for the final PCR reaction. The thermo cycling conditions for all the PCRs were as follows: 95 °C for 2 min, 95 °C for 15 s, 52 °C for 8 s, and 10 s at 72 °C for 30 cycles, with a final 2 min extension at 72 °C, and all the PCRs were performed in the MJ Research PTC-200 ThermalCycler. Amplified samples were evaluated by 1.5% agarose gel electrophoresis in Tris-borate-EDTA buffer and EtBr staining and illuminated by UV-transilluminator and documented by a gel documentation apparatus and1000bp DNA ladder was used as a marker for m-PCR assay. The expected size of products for *rfb*O157 and *flic*H7 genes amplification were 259 and 625 bp, respectively. Primers were listed in Table1.

Table 1. Primers used for detection of *E. coli* O157:H7

Primers	Primer sequence (5′ − 3′)	Product size (bp)	Reference
O157 Forward	CGGACATCCATGTGATATGG	259	(Paton and Paton, 1998)
Reverse	TTGCCTATGTACAGCTAATCC		
flic H7 Forward	GCGCTGTCGAGTTCTATCGAGC	625	(Gannon et al., 1997)
Reverse	CAACGGTGACTTTATCGCCATTCC		

RESULTS and DISCUSSION

Out of 120 traditional cheese samples 12 (10%) were determined as *E. coli*. The results showed that 7 (5.83%) of *E. coli* isolates from cheese were positive for O157:H7 (Table 2).

Among *E. coli* isolates, 3.33% were positive for *rfb*O157 and 0.83% were positive for *flic*H7 (Table 3).

Rahimi *et al* (2011) reported that 5 (4.2%) cheese samples were positive for *E. coli* O157 by PCR test. Abdul-Raouf *et al* (1996) reported that 6% of raw cow milk samples examined in Egypt were contaminated with *E. coli* O157:H7. Cheese containing unpasteurized milk was implicated as the vehicle of transmission in outbreaks of infectious intestinal disease (Altekruse et al., 1998). Incidence of *Escherichia coli* O157 in raw milk and white pickled cheese manufactured from raw milk in Turkey was studied. According to the results, *E. coli* O157 was determined in 1% of the total raw milk samples and in 4% of the cheese samples (Öksüz et al., 2004). Our findings were not differed greatly from other regions.

Table 2. Culture and API test results

Region	Samples	Positive samples by culture method	Positive samples by API test
Duhok center	40	2	2
Amedy	20	0	0
Zakho	28	2	1
Akre	32	8	4
Total	120	12	7

Table 3. PCR results for *E. coli* O157:H7

Samples	Positive samples from Biochemical tests	Positive samples from API test	*rfb*O157	flic H7	O157: H7
120	12 (10%)	7 (5.8%)	4 (3.33%)	1 (0.8%)	1 (0.8%)

The occurrence of *E. coli* in cheese samples may be due to lack of proper sanitation and absence of pasteurization of milk used for cheese production. To prevent the contamination of traditional cheese, strict hygienic procedures must be followed.

We can conclude that the routine microbiological examination should be done in producing factories, groceries and other food plants. Hygienic procedures should be applied for personnel whom involved in handling and preparing of food. Food safety information, training of staff, consumers should be organized and controlled by the governments and always work in partnership for eliminating food related EHEC illness.

REFERENCES

Abdul-Raouf, U.M., Ammar, M.S., Beuchat, L.R. 1996. Isolation of *Escherichia coli* O157:H7 from some Egyptian foods. International Journal of Food Microbiology, 29: 423-426.

Altekruse, S.F., Timbo, B.B., Mowbray, J.C., Bean, N.H., Potter, M.E. 1998. Cheese-associated outbreaks of human illness in the United States, 1972 to 1992: Sanitary manufacturing practices protect consumers. Journal of Food Protection, 61:1405-1407.

Andreoli, S.P., Trachtman, H., Acheson, D.W.K., Siegler, R.L., Obrig, T.G. 2002. Hemolytic uremic syndrome: epidemiology, pathophysiology, and therapy. Pediatric Nephrology, 17: 293-297.

Beutin, L., Krause, G., Zimmermann, S., Kaulfuss, S., Gleier, K. 2004. Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. Journal ofClinical Microbiology, 42: 1099-1108.

Blanco, J., Blanco, M., Blanco, J.E., Mora, A., González, E..A., Bernárdez, M.I., Alonso, M.P., Coira, A., Rodríguez, A., Rey, J., Alonso, J.M., Usera, M.A. 2003. Verotoxin-Producing *Escherichia coli* in Spain: Prevalence, Serotypes, and Virulence Genes of O157:H7 and non-O157 VTEC in Ruminants, Raw Beef Products, and Humans. Experimental Biology and Medicine, 228: 345-351.

Centers for Disease Control and Prevention (CDC). 2007 *Escherichia coli* O157:H7 infection associated with drinking raw milk- Washington and Oregon, November December 2005. Morbidity Mortality Weekly Report, 56: 165-167.

Feng, P., Weagant, S.D., Jinneman, K. 2011. Diarrheagenic *Escherichia coli.* FDA-BAM.

Ferens, W.A., Hovde, C.J. 2011. *Escherichia coli* O157:H7: Animal Reservoir and Sources of Human Infection. Foodborne Pathogens and Disease, 8: 465-487.

Gannon, V.P.J., D'souza, S., Graham, T., King, R.K., Rahn, K., Read, S. 1997. Use of flagellar H7 gene as a target in multiplex PCR assay and improved specificity and identification of enterohaemorrhagic *Escherichia coli* strains. Journal of Clinical Microbiology, 35: 656-662.

Govaris, A., Koidis, P., Papatheodorou, K. 2002. Behavior of *Escherichia coli* O157:H7 in sour milk, cow's milk yogurt and ewe's milk yogurt. Journal of Dairy Research, 69: 655-660.

Honish, L., Predy, G., Hislop, N., Chui, L., Kowalewska-Grochowska, K., Trottier, L., Kreplin, C., Zazulak, I. 2005. An outbreak of *Escherichia coli* O157:H7 hemorrhagic colitis associated with unpasteurized Gouda cheese. Canadian Journal of Public Health, 96: 182-184.

Karmali, M.A. 1989. Infection by verotoxin-producing *Escherichia coli*. Clinical Microbiology Reviews, 2: 15-38.

Morgan, D., Newman, C.P., Hutchinson, D.N., Walker, A.M., Rowe, B., Majid, F. 1993.Verotoxin producing *Escherichia coli* O157 infections associated with the consumption of yoghurt. Epidemiology and Infection, 111: 181-187.

Öksüz, Ö., Arici, M., Kurultay, S., Gümüş, T. 2004. Incidence of *Escherichia coli* O157 in raw milk and white pickled cheese manufactured from raw milk in Turkey. Food Control, 15, 453-456.

Paton, A.W., Paton, J.C. 1998. Detection and Characterization of Shiga Toxigenic *Escherichia coli* by Using Multiplex PCR Assays for stx1, stx2, eaeA, Enterohemorrhagic*E. coli*hlyA, rfbO111 and rfbO157. Journal of Clinical Microbiology, 36(2): 598-602.

Rahimi, E., Chaleshtori, S.S., Parsaei, P. 2011. Prevalence and antimicrobial resistance of *Escherichia coli* O157 isolated from traditional cheese, ice creamand yoghurt in Iran. African Journal of Microbiology Research, 5(22): 3706-3710.

Schlesser, J.E., Gerdes, R., Ravishankar, S., Madsen, K., Mowbray, J., Teo, A.Y.L. 2006. Survival of a five-strain cocktail of *Escherichia coli* O157:H7 during a 60 day aging period of cheddar cheese made from unpasteurized milk. Journal of Food Protection, 69: 90-99.

Schrade, J.P., Yager, J. 2001. Implication of milk and milk products in food disease in France and in different industrialized countries. International Journal of Food Microbiology, 67: 1-17.

Stampi, S., Caprioli, A., De Luca, G., Quaglio, P., Sacchetti, R., Zanetti, F. 2004. Detection of *Escherichia coli* O157 in bovine meat products in Northern Italy. International Journal of Food Microbiology, 90: 257-262.

Tobias, J., Vutukuru, S.R. 2012. Simple and rapid multiplex PCR for identification of the main human diarrheagenic*Escherichia coli.* Microbiological Research. 167 (9):564-570.